UNIVERSIDAD AUTÓNOMA DE MADRID

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IMPACTO DE LOS ULTRASONIDOS DE POTENCIA EN LA CALIDAD DE VEGETALES Y FRUTAS DURANTE EL PROCESO DE DESHIDRATACIÓN

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Tesis doctoral

Mayo 2013

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

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Memoria presentada por

JULIANA GAMBOA SANTOS

Para optar al grado de

Doctor

Directores:

Drs. Mª del Mar Villamiel Guerra y **Antonia Montilla Corredera**

Instituto de Investigación en Ciencias de la Alimentación

Consejo Superior de Investigaciones Científicas

Dª. Mª del Mar Villamiel Guerra, Dra. en Farmacia, Investigadora Científica del Instituto de Investigación en Ciencias de la Alimentación del C.S.I.C. y Dª. Antonia Montilla Corredera, Dra. Ingeniero Agrónomo, Científico Titular del Instituto de Investigación en Ciencias de la Alimentación del C.S.I.C.

CERTIFICAN: Que el presente trabajo titulado: **"Impacto de los ultrasonidos de potencia en la calidad de vegetales y frutas durante el proceso de deshidratación"**, y que constituye la memoria que presenta Dª. Juliana Gamboa Santos para optar al grado de Doctor por la Universidad Autónoma de Madrid, ha sido realizada en el Departamento de Bioactividad y Análisis de Alimentos del Instituto de Investigación en Ciencias de la Alimentación, bajo su dirección.

Y para que así conste firman el presente certificado en Madrid, a trece de mayo de 2013.

Fdo. Dª. Mª del Mar Villamiel Guerra

Fdo. Dª. Antonia Montilla Corredera

Agradecimientos

No resulta fácil resumir en un par de páginas lo que esta Tesis ha representado para mí. Sobre todo si reparo en todas aquellas personas que han influido y contribuido a lograr los objetivos (y no sólo académicos) que hoy tengo el gusto de celebrar...

Sin ustedes nada de esto hubiera sido posible...

En primer lugar me gustaría agradecer a mis directoras, la **Dra. Mar Villamiel** y la **Dra. Antonia Montilla** por dirigir mi trabajo con sabios consejos e infinita paciencia. Por la confianza que depositaron en mi (incluso antes de conocerme), haciéndome sentir, en todo momento, parte esencial de su equipo de trabajo.

Gracias al **Dr. Agustín Olano** y a la **Dra Nieves Corzo**, por abrirme las puertas de su grupo, por confiar en mí y por transmitirme su experiencia cada día, ayudándome en todo cuanto estuviera a su alcance.

Gracias al **Consejo Superior de Investigaciones Científicas (CSIC)** por la concesión de la beca JAE, sin la cual no me hubiera sido posible desarrollar este trabajo.

Gracias a la **Dra. Lourdes Amigo**, directora del antiguo Instituto de Fermentaciones Industriales (CSIC) y a la **Dra. Victoria Moreno-Arribas**, directora del actual Instituto de Investigación en Ciencias de la Alimentación (CIAL, CSIC-UAM), por facilitarme la inclusión en los Centros a su cargo y por su apoyo constante durante todo el período de realización de mi Tesis doctoral.

Gracias a la **Universidad Autónoma de Madrid (UAM)** y, en especial, al **Dr. Guillermo Reglero** por su ayuda como tutor de este trabajo.

Asimismo, agradezco al **Dr. Enrique Riera** por su colaboración en la realización de los procesos de deshidratación de zanahorias con ultrasonidos de potencia, en el Instituto de Acústica. Gracias, además, por transmitirme sus conocimientos sobre las bases físicas, los mecanismos y los efectos de los ultrasonidos.

Agradezco, de modo especial, al **Dr. Antonio Mulet**, por acogerme como una integrante más en su grupo de investigación en Análisis y Simulación de Procesos Agroalimentarios (ASPA), durante mi estancia en la Universidad Politécnica de Valencia (UPV). Asimismo, al **Dr. José Vicente García Pérez** y al **Ing. Juan Andrés Cárcel**,

Agradecimientos

por la ayuda y excelente predisposición durante la realización de los secados de fresa asistidos por ultrasonidos de potencia. Como también, agradezco a todos los integrantes de ASPA, que han contribuido a que recuerde a Valencia con mucho cariño.

Quisiera agradecer además al **Dr. Javier Moreno**, a la **Dra. Mª Dolores del Castillo** y a la **Dra. Tiziana Fornari**, por siempre estar dispuestos a brindarme su ayuda y conocimientos.

Además, quisiera agradecer en especial a mis compañeros y amigos *del labo*, mejor dicho: *a **mi familia en España***. A los del principio y a los del final que tuve la suerte de encontrar en esta carrera hacia la Tesis. En primer lugar **a Cris** quien me mostró, con una sonrisa, la esencia del laboratorio, enseñándome "casi" todo cuanto he aprendido. A **Alejandra**, que me involucró en el grupo cuando estaba recién llegada, me ofreció su casa, me presentó a sus amigos e impidió las "morriñas" de los primeros meses. A **Valle** y **Milhou**, por ser mis hermanos mayores los primeros tiempos, por acarrear muebles del IKEA conmigo y ofrecerme todo y cuanto podían desde el minuto cero. A **Myriam**, **José Manuel** y **Fernando**, tres ilustres sabios que conocí ya al final de su período de tesis. Me llevo de ellos talento y risas a raudales...

A **Martita**, amiga, hermana, compañera de tantas aventuras (y miles de centilitros del brebaje de los dioses), memorables charlas, risas y cuasi-llantos a veces...Gracias por tu empuje, tus ánimos y, sobre todo, por *tu amistad*. *¡No olvides que siempre habrá un buen sitio para reencontrarnos!* A **Ana Belén**, mi codo a codo en los primeros años, amiga, cómplice, confesionario vivo *¡gracias por tantos buenos ratos compartidos!* A mi compañera y amiga **Moni**, de Zaragoza, otra esmeralda en bruto en la Madre Patria y *pareja de pádel como pocas*...je. A **Ana Ruiz**, por su ayuda, infinita predisposición y obviamente por su amistad y necesaria compañía sobre todo en los últimos y duros momentos. A **Paulina**, mi amiga valenciana, que impresiona a quienquiera con su desparpajo y entusiasmo. *¡Gracias por tantos buenos ratos!* A "mi" **Robert**, compañero de vitaminas, rehidrataciones y tantas otras yerbas (sabemos que nuestra relación no empezó con buen pie pero ahora se que tengo un amigo de Navalucillos por el mundo con quien compartir una grata charla). *Gracias, Robert*, por aportar varios granitos de arena a mi tesis y sobre todo por tantos ánimos en las etapas de bajón. A **Montse**, otra amiga sureña, por tantas charlas y ocasiones memorables. Deseo que tu gaussiana del comportamiento esté, de ahora en más, en la cresta de la ola...jeje.

Agradecimientos

A mi mano derecha en el “laboratorio H”, **Marinilla**, por acompañar el trance de la escritura con palabras de aliento, tantos *See you tomorrow* y por ser mi compañera de piso de día (prometo dejarte la casa en orden jaja). A las chicas del E, compañeras de noches y días, sobre todo en el último tiempo: la **Bea** y la **Nuri**, gracias por la buena onda anti-stress.

A todos, que fueron varios, los transeúntes que dejaron marcas en el Labo y que recordaré siempre con mucho cariño: a **Alejandro, Lucía, la Vane, Iratxe, Laura, Sara, Florencia, Ángel**...por iluminarnos con el toque “más jóven” el día a día y, en algún caso, por darme la posibilidad de seguir aprendiendo *“de las dudas”* (mías y suyas).

A los chicos y chicas “del CIAL”: **Gussiel, Lydia, Dani, Gustavo, Paqui, Consti, Almu, Jordan, David, Jose, Isidoro, Eli, Rosi, Carmen, Azu *et al***...entre otros muchos, por ser parte de mis “despejes”, por las cañitas y las charlas de pasillo “a las apuradas”...

A **Sonia**, **Laura** y **Cipri** del laboratorio de Química Orgánica de República Argentina y aledaños, cómo olvidar tantas carcajadas compartidas, insuperables risas y buenos momentos entre pasillos y neveras.

Last but not least, a **Oswaldo**, mención especial, como hermano latinoamericano que es, por su apoyo incondicional no sólo en el trabajo sino fuera de él. He encontrado un hermano del corazón en España. Incontables risas, historias y complicidades nos unen. *¡Mil gracias my friend!* Seguiremos en contacto vaya a saber desde qué lugares remotos del planeta Tierra.

Amigos todos, las circunstancias de la vida nos seguirán separando pero saben bien que *yo no me olvidaré de ustedes*. Es más, los invocaré cuando haga falta y **siempre podremos encontrarnos a mitad de camino**.

Agradecimientos

Finalmente, quisiera dedicar no sólo esta tesis, sino también esta etapa de mi vida, **a mi familia** que día a día han sabido *estar conmigo* a pesar del océano de distancia que nos separaba. A todos ellos, que me extrañaron mil veces, pero a la vez me incentivaron a seguir, a completar este camino sinuoso pero excitante.

A mis padres, que me inculcaron el esfuerzo, el espíritu crítico e investigador, que me animaron desde pequeña a hacer lo que quería hacer (*y, de hecho, me regalaron una calculadora a los 4 años…je je*). Que no demostraron ni una pizca de miedo e inseguridad ante mi partida, que superaron los obstáculos y lo reflejaron a cada paso *¡Ahora ya puedo decir que soy doctora como ustedes!*

A mis hermanos, **Pablo y Flor**, que mantuvieron el barco a flote tantas veces, que me impresionaron con su sensibilidad, con su comprensión, con su sabiduría. Gracias por los abrazos en los momentos justos, por las palabras de aliento y *perdón por las muchas ausencias*. *¡Ya lo recuperaremos con creces y tendrán que aguantarme!*

A mi **abuela Bocha**, que soportó tantos "temblequeos de pera", contuvo tantas lágrimas en cada una de mis "vueltas a Madrid", que se alegró tantas veces por mis e-mails y por verme superar etapas. ELLA, que se amigó con la tecnología (y tanto más!) con tal de comunicarse y estar ahí para cuando sus nietos necesitaran unas palabras de abuela. *Gracias abula por estar siempre.*

A **mis abuelos** que no están (**Lía, Ruben y Caíto**) por pasarme estos genes preciosos que los involucran en cuanto hago y, junto a la Abula, son los pilares de esta familia que atravesaron vaya a saber cuántos caminos más sinuosos que este.

A mis tíos y tías. En especial a mis **Tías Marce y Ana** por ser más que eso. Por demostrar cada una desde su lugar que podía confiar en ellas. Por ser cómplices, testigos y hacerme reír mil veces ante la adversidad…en tiempos del reino de Camboya (je je).

A **mis primos (Santi, Magui, Ale y Caro)**, pares, hermanos, amigos, por esos domingos de necesaria contención familiar, por tantas fiestas, tantas birras y asados al sol en épocas de quinta.

Agradecimientos

A mis amigos de Argentina que extrañé cada día, en cada mate en solitario, con cada charla profunda, con cada foto, en cada cumpleaños que pasaba a la distancia: **Marian, Juan Martín (el pela), Juan Ma, el Negro, July, Anita, Naty, Barbi, Teté, Guada, Gise, Gerald, Mari, Juli Altabegoiti *et al***. Además no quisiera dejar de mencionar con carácter especial a mis amigos *del alma*: **Luck y Mache**, que además de estar al pie del cañón ante cualquier llamado de emergencia han venido a Madrid (desde Sudáfrica y Argentina, respectivamente) a hacerme compañía y amenizar los momentos difíciles (y cayeron justo para la ocasión…je). A todos ellos: *¡Lo nuestro es para toda la vida!* Ya recuperaremos el tiempo perdido y seguiremos ampliando la familia…

Por último, a mis compañeros en la vida madrileña, a **Mária** por aguantar "casi" todo el tiempo de Tesis, por soportar mis caras largas, mi carácter a veces difícil y mi espíritu cansado otras tantas. Pero, sobre todo, *por enseñarme a querer a los gatos (je je)*. Finalmente a **Noe**, mi bello hijo de otra especie, quien se ha pasado horas a mi lado durante la escritura, sin apenas decir *miau*…

¡Gracias a todos!

I. ABREVIATURAS/ABREVIATIONS

2-FM-AA: 2-furoilmetil aminoácidos

2-FM-Arg: 2-furoilmetil arginina

2-FM-GABA: 2-furoilmetil ácido gamma aminobutírico

2-FM-Lys: 2-furoilmetil lisina (furosina)

ANOVA: análisis de varianza

API-ES positive: Atmospheric Pressure and positive polarity

a_w: actividad de agua

CCD: Central Composite Design

CFU: Colony Forming Units

DE: Desviación Estándar

D_e: difusividad efectiva de materia

DM: Dry Matter

DO: Deshidratación Osmótica

DS-MS: Direct Sampling-Mass Spectrometry

DTT: dithiothreitol

E_a: Energía de activación

***ER*:** External Resistance

FD: Freeze Drying/Freeze Dried

FID: Flame Ionization Detector

FO: Función Objetivo

FOS: fructooligosacáridos

GAE: Gallic Acids Equivalents

GC-FID: Gas Chromatography-Flame Ionization Detector

HMF: hidroximetilfurfural

HPLC: High-Performance Liquid Chromatography

HTST: tratamientos de alta temperatura, tiempos cortos

***k*:** coeficiente externo de transferencia de materia

LC-MS: Liquid Chromatography-Mass Spectrometry

LL: Leaching Loss

LSD: mínima diferencia significativa

LTLT: tratamientos de baja temperatura, tiempos largos

MR: Maillard Reaction

MRE: Mean Relative Error

MS e-nose: Mass Spectrometry electronic-nose

MS: Material Seca

ORAC: Oxygen Radical Absorbance Capacity

PME: pectinmetilesterasa

POD: peroxidasa

RE: Resistencia Externa

***RI*:** Resistencia Interna

RM: Reacción de Maillard

RP-HPLC: Reverse Phase High-Performance Liquid Chromatography

RP-HPLC-DAD: Reverse Phase High-Performance Liquid Chromatography–Diode Array Detector

RP-HPLC-UV: Reverse Phase High-Performance Liquid Chromatography-UV/Vis detector

RR: Rehydration Ratio

RSM: Response Surface Methodology

SD: Standard Deviation

SDS-PAGE: electroforesis en gel de poliacrilamida con dodecilsulfato sódico

SRE: Sin Resistencia Externa

TE: Trolox Equivalent

TMSO: Trimetilsilil-Oximas

TN: contenido en Nitrógeno Total

TPC: contenido de polifenoles totales

UFC: Unidades Formadoras de Colonias

US: ultrasonidos

VAR: explained variance

II. ÍNDICE DE TABLAS Y FIGURAS

TABLAS/TABLES

FIGURAS/FIGURES

III. RESUMEN

En la presente Tesis se ha estudiado la influencia de los ultrasonidos (US) de potencia en el proceso de deshidratación de vegetales y frutas, tanto en el pre-tratamiento como en el secado, empleándose diversos parámetros de calidad. Los indicadores químicos seleccionados han sido las enzimas peroxidasa y pectinmetil esterasa, la vitamina C, los carbohidratos, las proteínas, los polifenoles y los 2-furoilmetil aminoácidos (indicadores de las etapas iniciales de la reacción de Maillard); además, se han evaluado la capacidad de rehidratación, las pérdidas por lixiviado y el encogimiento, así como las características organolépticas del producto final. En primer lugar, con objeto de conocer la calidad de este tipo de productos de los que dispone el consumidor, se analizaron muestras industriales y comerciales de vegetales y frutas ampliamente consumidos en la actualidad. Además, con fines comparativos, se llevaron a cabo tratamientos de secado en un prototipo por convección cuyas condiciones de procesado se optimizaron para obtener productos de alta calidad. Posteriormente, se estudió la viabilidad de la aplicación de los US de potencia como tratamiento previo al secado convectivo, encontrándose efectos similares en lo referente a pérdidas por lixiviado e inactivación enzimática en los tratamientos convencionales a baja temperatura y largo tiempo y en los tratamientos con sonda de US, pero con una significativa reducción en el tiempo con estos últimos. Finalmente, la aplicación de US en el secado, en concreto de zanahoria y fresa, produjo reducciones significativas en el tiempo de procesado, así como productos finales de elevada calidad. Dicha calidad fue superior a la de productos comerciales y superior o equivalente a la de muestras obtenidas en similares condiciones en un prototipo de secado convectivo, e incluso, en el caso de algunos indicadores, semejante a la de muestras liofilizadas. Los resultados hallados en esta Tesis constituyen un avance importante en la aplicación de US de potencia para obtener, de modo eficiente, vegetales y frutas de alta calidad y bioactividad que respondan a las demandas del consumidor actual.

ABSTRACT

In this Thesis the influence of power ultrasound (US) on the dehydration process of fruits and vegetables both in pre-treatment and drying was studied. Various quality parameters were used. Chemical indicators selected were: pectinmethyl esterase and peroxidase enzymes, vitamin C, carbohydrates, proteins, polyphenols and 2-furoylmethyl amino acids (indicators of the early stages of the Maillard reaction); in addition, physical properties were evaluated based on rehydration capacity, leaching losses and shrinkage, as well as the organoleptic characteristics of the final product were also evaluated. Firstly, in order to know the quality of this type of products, available to the consumer, industrial and marketed samples of fruits and vegetables widely consumed today were analysed. Furthermore, for comparison purposes, drying treatments were conducted in a prototype convection drier, the processing conditions of which were optimised to obtain high quality products. The feasibility of application of power US as a treatment prior to convection drying was subsequently studied. Similar effects with regard to leaching losses and enzyme inactivation were found in conventional low temperature and prolonged treatments and in treatments with a US probe, but with a significant reduction in the time of the latter. Finally, application of US in drying, in particular of carrots and strawberries, produced significant reductions in processing time and high quality end-products. The quality mentioned was superior to that of marketed products and superior or equivalent to samples obtained under similar conditions in a prototype convection drier and, in the case of some indicators, even similar to that of freeze-dried samples. Results found in this Thesis constitute a major advance in the application of power US for efficiently obtaining fruits and vegetables, with high quality and bioactivity that meet the demands of today's consumers.

IV. ESTRUCTURA DE LA MEMORIA

La presente memoria está estructurada en las seis secciones que se indican a continuación:

Introducción general: donde se introduce al lector en la deshidratación de vegetales y frutas, detallando los antecedentes existentes relacionados con los objetivos planteados para esta Memoria.

Justificación y objetivos: donde se justifica la importancia del tema de estudio, su contexto actual y los objetivos generales y parciales planteados para este trabajo.

Plan de trabajo: donde se explica de forma global y esquematizada cómo se ha abordado el trabajo para alcanzar los objetivos prefijados.

Resultados y discusión: esta sección está dividida en tres sub-secciones, ordenadas por temáticas. En cada sub-sección un prefacio resume su contenido haciendo énfasis en los resultados más notables. A continuación, se exponen los trabajos científicos generados (publicados, enviados o pendientes de publicación), en lengua inglesa y de acuerdo al formato convencional de las publicaciones científicas (Resumen, Introducción, Materiales y Métodos, Resultados y Discusión). La sección 4.1 se centra en los procesos convencionales utilizados para deshidratar vegetales y frutas en la industria, estudiándose productos comerciales y los obtenidos en un prototipo de secado por convección. Los resultados de la primera sección han generado cuatro artículos (dos de ellos pendientes de publicación). En la sección 4.2 se estudian en zanahoria pre-tratamientos convencionales y con aplicación de US de potencia y su efecto en el posterior secado por convección. Esta sección ha dado lugar a tres publicaciones. La última sección (4.3) se ha enfocado a la aplicación de US de potencia en el secado convectivo de zanahoria y fresa, considerando tanto la cinética de deshidratación como los parámetros de calidad químicos y físicos más relevantes en los productos deshidratados. Esta sección ha generado tres trabajos científicos, dos de ellos pendientes de publicación.

Discusión general: donde se persigue unificar e hilar los resultados conseguidos en cada una de las secciones de esta Memoria. De modo general

se discuten los resultados obtenidos por otros autores y la relevancia de nuestros hallazgos, destacando la viabilidad de la aplicación de los US a lo largo del proceso de secado.

Conclusiones generales: donde se presentan las conclusiones más relevantes obtenidas para todos los trabajos expuestos en la sección de Resultados.

Introducción general

1. INTRODUCCIÓN GENERAL

A lo largo de los años, los hábitos alimenticios de la sociedad se han ido modificando, tanto por el tipo de alimentos que se consumen como por el tiempo dedicado a su preparación. El ritmo de vida actual ha propiciado el auge de alimentos que resultan prácticos para el consumo, pero que aportan escaso valor nutritivo. Estas tendencias han contribuido, sin lugar a dudas, a incrementar la incidencia de ciertas enfermedades relacionadas con una inadecuada alimentación, destacando, entre otras, la obesidad, de especial relevancia en la etapa infantil (WHO, 2010; Mesas y col., 2012). Las recomendaciones de las autoridades sanitarias y la conciencia de los riesgos de hábitos alimenticios inadecuados están empezando a tener repercusión en los consumidores, quiénes eligen un alimento no sólo por la comodidad y el tiempo que supone su preparación, sino también por los efectos que pueden ejercer sobre su salud, sin descuidar las características organolépticas y el valor nutritivo de los mismos. Una de las tendencias actuales es incrementar el consumo de vegetales y frutas y disminuir la ingesta de alimentos con aportes excesivos de grasas y azúcares, con contenidos elevados de calorías y sin aporte nutritivo. Así, de acuerdo con Basu y col. (2010), es previsible que, por ejemplo, en EEUU incremente el consumo de frutas entre 24-27% desde el año 2000 hasta el 2020.

1.1. Composición y propiedades de vegetales y frutas. Zanahoria y fresa

Estudios experimentales y epidemiológicos han demostrado que el consumo de vegetales y frutas resulta beneficioso para la salud humana no sólo por el aporte en constituyentes con elevado valor nutritivo, sino también porque puede disminuir el riesgo de padecer determinadas enfermedades tales como cáncer, diabetes y patologías cardiovasculares, entre otras, además de retrasar procesos degenerativos, incluyendo el envejecimiento. Estos efectos se encuentran asociados a constituyentes biológicamente activos que se hallan presentes de modo natural en los vegetales y las frutas, siendo los más importantes los compuestos fenólicos, los carotenoides, las

vitaminas (C y E, entre otras) y la fibra (Fenoll y col., 2011; Sablani y col., 2011; Landete y col., 2012).

Entre los vegetales, cabe destacar la zanahoria (*Daucus carota* L.) por su elevado contenido en ß-caroteno (6,4-8,3 mg/100 g), complejo vitamínico B, vitamina C (2,6-5,9 mg/100 g) y minerales (USDA, 2013). El ß-caroteno, es un precursor de la vitamina A que sólo se encuentra como tal en productos de origen animal. En las zanahorias, constituye el carotenoide predominante y está localizado en los cromoplastos en forma de cristales estabilizados por lipoproteínas. Los humanos son capaces de convertir fácilmente el ß-caroteno en vitamina A, siendo este aspecto de gran importancia, dado que su carencia podría implicar ceguera, perturbaciones en el desarrollo normal de huesos y dientes, trastornos en células epiteliales y mucosas de la nariz, garganta u ojos que pueden reducir la resistencia a las infecciones (Potter y Hotchkiss, 1995).

Otros compuestos de interés en zanahoria son los polifenoles los cuales destacan tanto por sus propiedades antioxidantes como antibacterianas, anticarcinogénicas y vasodilatadoras (Gonçalves y col., 2010). Además, se ha estudiado el papel de los polifenoles en la prevención de enfermedades neurodegenerativas, como el Alzheimer y el Parkinson (Canturi-Castelvetri y col., 2000; Halliwell, 2001), y en el tratamiento de la diabetes (Zunino y col., 2007) y la osteoporosis (Atmaca y col., 2008; Ma y col., 2008), entre otras patologías. La presencia de compuestos fenólicos en zanahoria contribuye, además, a su calidad sensorial en el color (Zhang y col., 2005), el sabor ligeramente amargo (Kreutzman y col., 2008) y el aroma (Naczk y Shahidi, 2003).

La composición química de la zanahoria referente a los constituyentes mayoritarios se recoge en la **Tabla 1.1** (Belitz y col., 2009a). El agua, como en el resto de vegetales, representa la mayor parte. Dentro de los sólidos, los carbohidratos son mayoritarios, siendo los solubles los que predominan con contenidos en glucosa, fructosa y sacarosa entre 0,59-1,04; 0,55-1,00 y 2,70-3,59 g/100 g, respectivamente, dependiendo de la variedad y del grado de madurez del vegetal (Gajewski y col., 2009). Entre los carbohidratos solubles minoritarios destacan *mio*-inositol, *scyllo*-inositol y sedoheptulosa (D-*altro*-2-heptulosa), estos últimos identificados recientemente (Soria y col., 2009a). Además, el contenido final de carbohidratos puede variar según los

distintos procesamientos y condiciones de almacenamiento a los que se ha sometido la hortaliza (Nyman y col., 2005).

Tabla 1.1 Composición química de la zanahoria

Constituyentes mayoritarios	Composición* (g/100 g de producto comestible)
Agua	88,2
Carbohidratos solubles	4,8
Fibra	3,6
Proteínas	1,1
Lípidos	0,2
Cenizas	0,8

*Valores medios (Belitz y col. 2009a)

En España, en 2006, la producción de zanahorias alcanzó las 600 mil toneladas, ocupando el quinto puesto a nivel europeo detrás de Polonia y Reino Unido (833 mil toneladas), Francia (693 mil toneladas) e Italia (615 mil toneladas) (Belitz y col., 2009a).

Por lo que se refiere a las frutas, la fresa (*Fragasia x annanasa*) destaca por su palatabilidad y riqueza en componentes bioactivos. Esta fruta, debido a su bajo valor calórico (32 kcal/100 g) (por su moderada concentración de azúcares) y alto contenido en ácido fólico, antioxidantes (vitamina C, flavonoides y antocianinas), potasio y salicilatos, está especialmente recomendada en dietas de adelgazamiento y prevención de ciertas enfermedades. A pesar de su bajo contenido lipídico, resulta una fuente interesante de ácidos grasos esenciales, mayormente poli-insaturados (alrededor de un 72%) (Giampieri y col., 2012). Sin embargo, el elevado contenido en vitamina C (58,8 ± 2,5 mg/100 g; USDA, 2013) es, quizás, el factor más determinante para ser considerada como una de las frutas más apreciadas.

La vitamina C se encuentra en todas las células animales y vegetales en forma libre y, probablemente, unida a proteínas. Muchas especies animales sintetizan la vitamina C, siendo los humanos una de las excepciones. Nuestra especie es incapaz de elaborar la enzima L-gulonolactona oxidasa, responsable de la síntesis de la vitamina C. Por lo tanto, es indispensable su consumo, dado que dicha vitamina no sólo previene enfermedades como el

escorbuto, sino que además tiene un rol muy importante como antioxidante biológico (Santos y Silva, 2008). En fresas se atribuye a esta vitamina hasta un 30% de la capacidad antioxidante total de esta fruta (Giampieri y col., 2012). Varios estudios relacionan el consumo de vitamina C con la prevención y el tratamiento de enfermedades coronarias (Lee y Kader, 2000), cáncer (Du y col., 2009), enfermedades mentales, infertilidad y SIDA (Dadali y Özbek, 2009).

En general, el ácido ascórbico constituye el 90% del total de la vitamina C en vegetales y frutas (Agar, 1995). Se ha comprobado que el ácido ascórbico previene el cáncer por su capacidad para inhibir la formación de óxido nitroso en el estómago y estimular el sistema inmune (Byers y Perry, 1992; Du y col., 2009). Asimismo, aumenta la disponibilidad del yodo (Gowri y col., 2001) y su deficiencia puede provocar fragilidad capilar, hemorragias en las encías, debilidad en los dientes y trastornos de las articulaciones (Potter y Hotchkiss, 1995).

Con respecto a los polifenoles de la fresa destacan las antocianinas, responsables del color rojo propio de este fruta, los flavonoles y los derivados de ácido hidroxicinámico y elágico (Wojdylo y col., 2009). Como se ha indicado anteriormente, los polifenoles son unos de los constituyentes claves en frutas y vegetales por su efecto positivo en la salud humana.

Según se refleja en la **Tabla 1.2**, los carbohidratos son los componentes más abundantes. De ellos, la fructosa (2,44 g/100 g) representa el 50%, completando el total la glucosa (1,99 g/100 g) y la sacarosa (0,49 g/100 g) (Giampieri y col. 2012).

Tabla 1.2 Composición química de la fresa

Constituyentes mayoritarios	Composición* (g/100 g de producto comestible)
Agua	91,0
Carbohidratos solubles	4,9
Fibra	2,0
Proteínas	0,7
Lípidos	0,3
Cenizas	0,4

*Composición media (Giampieri y col. 2012)

La fresa es considerada una de las frutas de temporada más consumida. En el año 2006, la producción mundial superó los 4 millones de toneladas, siendo Europa el mayor productor (alrededor de 1,5 millones de toneladas) (Belitz y col., 2009b). Cabe destacar que, para el mismo año, España ocupó el segundo lugar como país productor de fresa con 334 mil toneladas, detrás de Estados Unidos cuya producción anual superó ampliamente el millón de toneladas.

1.1.1. El mercado de vegetales y frutas deshidratados

A pesar de las ventajas del consumo de vegetales y frutas, su elevado contenido en agua (superior al 80%) y su carácter estacional, hace necesario su procesamiento o transformación para ampliar su periodo de vida útil, disponibilidad y utilizar excedentes. Entre los diferentes procesos existentes, la deshidratación es, probablemente, el método más antiguo y extendido para conservar alimentos. La eliminación del agua contenida en los alimentos, hasta valores de extracto seco de 82 a 85%, previene la proliferación de microorganismos responsables del deterioro y minimiza las reacciones indeseables que se producen en condiciones de elevada actividad de agua (a_w), puesto que en los alimentos deshidratados suele estar por debajo de 0,3 (Belitz y col., 2009a). Además, reduce sustancialmente el volumen y el peso de los alimentos, facilitando su transporte y almacenamiento durante largos períodos de tiempo.

En la mayoría de los países, el mercado de vegetales y frutas deshidratados es de una importancia considerable. Su demanda ha experimentado un importante auge en los últimos años, tendencia que es de

esperar continúe en los próximos años en las economías emergentes (Satyanarayan y Raghavan, 2012). Actualmente, los vegetales deshidratados se utilizan como ingredientes culinarios o bien formando parte de platos precocinados de fácil consumo. El mercado ofrece además una amplia gama de vegetales y, especialmente, frutas deshidratadas que pueden consumirse como "snacks", o bien rehidratarse. En este sentido, por ejemplo, la fresa es comúnmente utilizada como ingrediente en diversos productos, tales como helados, mermeladas, yogures, barritas de cereal y tartas, entre otros.

El mercado europeo estimó su producción de vegetales deshidratados en 900 mil toneladas, con un valor de 6.000 millones de euros en el año 2000 (Torringa y col., 2001). Con respecto a las frutas deshidratadas, en el año 2006, la producción en la UE fue de 428 mil toneladas, mientras que el consumo fue de 871 mil toneladas. España, en ese año, fue el tercer productor europeo de frutas deshidratadas, con un consumo aparente (considerando la producción, importación y exportación) de 82 mil toneladas (CBI Market Survey, 2008). Las frutas de consumo preferente fueron: dátiles, ciruelas y manzanas y, en menor medida, higos y albaricoques. Un aspecto a señalar es que las importaciones de frutas deshidratadas en España, para el año 2006, representaron casi el 50% del consumo; esto indica que la producción local tiene un gran potencial de crecimiento para satisfacer la demanda interna.

Con la finalidad de atender a la demanda de productos deshidratados, tanto desde el ámbito de la empresa como de la investigación se ha impulsado la mejora de los procesos convencionales y la búsqueda de otros nuevos que amplíen la vida útil de los productos sin detrimento de su calidad nutritiva, pero que además diversifique la oferta de cara al consumidor. En este sentido, existen numerosos trabajos enfocados al estudio sobre diferentes procesos de deshidratación y pre-tratamientos, así como sobre su posible efecto en la calidad del producto final.

1.2. Tratamientos previos a la deshidratación de vegetales y frutas

1.2.1. Convencionales

Como paso previo a la deshidratación, se pueden realizar pre-tratamientos, siendo el escaldado uno de los más aplicados en el caso de los vegetales. En el escaldado convencional la materia prima vegetal se somete a la acción del vapor o se sumerge en agua en condiciones controladas de tiempo y temperatura. El escaldado puede realizarse a bajas temperaturas (50–70 ºC) durante tiempos prolongados (hasta 1 h) (LTLT) o temperaturas elevadas (cercanas a la de ebullición o en vapor) durante tiempos cortos (HTST) (Lewicki, 2006). El escaldado por vapor se utiliza, principalmente, en alimentos de gran superficie relativa, debido a que las pérdidas de nutrientes por lixiviado se reducen considerablemente, comparadas con el escaldado en agua caliente (Fellows, 1994). El tratamiento LTLT, en general, produce mejoras en la textura del vegetal, debido a un menor encogimiento durante el secado y a una textura más firme una vez rehidratado (Lewicki, 2006).

En general, los tratamientos HTST, reducen considerablemente los tiempos de secado, lo cual, puede traducirse en una reducción del coste tanto energético como operativo, así como en la obtención de productos de mayor calidad. Así, por ejemplo, Górnicki y Kaleta (2007) obtuvieron una reducción del 13% en los tiempos de secado posterior en muestras de cubos de zanahoria escaldadas en agua hirviendo durante 6 min respecto a muestras sin escaldar. La aceleración del proceso de secado debido al pre-tratamiento tiene su fundamento en la aparición de microfisuras y apertura de poros en el tejido vegetal, que favorecen los fenómenos de transferencia de agua a través de la matriz vegetal (Santos y Silva, 2008).

Aunque los pre-tratamientos convencionales más extendidos utilicen agua hirviendo o vapor, existen otras variantes como la deshidratación osmótica (DO), que se emplea ampliamente en frutas y consiste en la inmersión del alimento en disoluciones azucaradas (Arballo y col., 2012) o salinas en el caso de vegetales (Kurozawa y col., 2012). La DO permite la reducción de la a_w, obteniéndose productos con características sensoriales atrayentes. Sin embargo, debido al intercambio de solutos entre la disolución

y el alimento, se generan nuevos sabores en el producto final. Las frutas obtenidas mediante este método son semielaboradas, presentando una humedad intermedia, por lo que, para prolongar la vida útil, requieren un tratamiento posterior, siendo el secado una de las opciones más empleadas (Rastogi y col. 2002; Contreras y col. 2007).

1.2.1.1. Efectos del escaldado en los constituyentes

El objetivo principal del escaldado es reducir el número inicial de microorganismos, inactivar enzimas y retardar o interrumpir las reacciones oxidativas, elevando la calidad a la vez de preservar las características organolépticas (Rahman, 2003; Doymaz, 2008a). Sin embargo, es preciso tener en cuenta que en algunos vegetales y frutas más sensibles, el pre-tratamiento puede dar lugar a importantes pérdidas por lixiviado de vitaminas hidrosolubles, polifenoles, minerales y azúcares de bajo peso molecular, y modificaciones de la estructura celular, por lo que no siempre resulta recomendable llevarlo a cabo.

1.2.1.1.1. Inactivación enzimática

Entre las enzimas que se vinculan con modificaciones negativas en los vegetales y frutas, la peroxidasa (POD) es una de las más importantes. Este enzima cataliza reacciones de óxido-reducción y puede provocar cambios en el color y flavor de los vegetales y las frutas. La inactivación de la POD durante el pre-tratamiento incrementa la vida útil de los productos, siendo su determinación frecuentemente utilizada como índice de la eficacia del escaldado debido a su termorresistencia (Cruz y col., 2006; Polata y col., 2009). Sin embargo, se ha visto que no es necesaria la completa inactivación de la POD para preservar la calidad en vegetales congelados (Baardseth, 1978) y un 5% de actividad residual puede no llegar a afectar a la calidad de estos vegetales durante su almacenamiento (Baardseth y Slinde, 1981). La cinética de inactivación de la POD en zanahoria ha sido estudiada por Soysal y Söylemez (2005) quienes indicaron la existencia de dos isoformas, resistente y lábil, a temperaturas de 35 a 65 ºC, durante tratamientos térmicos de hasta 180 min. Lemmens y col. (2009) obtuvieron actividades

residuales de la POD del 70% tras un escaldado de zanahorias en agua a 60 ºC, durante 40 min (LTLT).

Por otro lado, otro aspecto importante de los tejidos vegetales es su estructura que depende de la integridad de su pared celular, siendo las sustancias pécticas los constituyentes que le confieren firmeza y elasticidad (Day y col., 2012). En este sentido diversos estudios han comprobado que la pectinmetilesterasa (PME) tiene un papel primordial en los cambios de textura observados en vegetales sin escaldar o escaldados en condiciones LTLT (Lemmens y col., 2009). La PME actúa sobre la pectina metoxilada liberando metanol y moléculas de pectina con un menor grado de esterificación, lo cual conduce a un mayor entrecruzamiento entre éstas y los cationes divalentes tales como el calcio y el magnesio, incrementando la firmeza del tejido (Sanjuán y col., 2005; Lemmens y col., 2009). Este cambio estructural evita el daño térmico posterior, aunque la capacidad de rehidratación puede disminuir (Quintera-Ramos y col., 1998).

Fraeye y col. (2009) propusieron una combinación de adición de PME de *Aspergillus aculeatus* y cationes Ca^{2+} para mejorar la textura de fresas sometidas a tratamientos térmicos en agua a 95 ºC (10 a 40 min). De sus resultados concluyeron que este tratamiento incrementaba tanto la retención de materia como la firmeza de la fruta durante el procesamiento, a la vez que limitaba la pérdida de pectinas.

1.2.1.1.2. Pérdidas por lixiviado

Como se ha indicado, otro efecto importante que se puede producir durante el escaldado son las pérdidas por lixiviado. Neri y col. (2011) obtuvieron reducciones apreciables, tanto de peso como de materia seca, en zanahorias sometidas a diferentes tratamientos en agua (75 y 90 ºC, 3 y 10 min). En todos los casos, las pérdidas fueron atribuidas al daño térmico ocasionado en el tejido vegetal que redujo la resistencia a la transferencia de masa y, por tanto, favoreció el lixiviado de sólidos desde la matriz sólida hacia el agua de escaldado.

Las pérdidas de sólidos producidas durante el tratamiento de escaldado suelen corresponder mayoritariamente a los carbohidratos (Mayer-Miebach y Spies, 2003) y dependiendo de la intensidad del tratamiento puede afectar a

las características organolépticas del producto. Inyang y Ike (1998) observaron una leve disminución de carbohidratos en frutos típicos africanos (okra) sometidos a tratamientos de escaldado en agua (98 ºC durante 3 min). Wennberg y col. (2006) mostraron pérdidas de 30-34% de extracto seco en repollos escaldados en ebullición durante 5 min, de las cuales entre 82 y 90% fue debido a la pérdida de carbohidratos de bajo peso molecular.

Junto con los carbohidratos, los volátiles constituyen otros de los compuestos implicados en el sabor de los vegetales, siendo de los factores más influyentes en su aceptación por parte del consumidor (Rosenfeld y col., 2002). Son también altamente sensibles a los tratamientos de escaldado. Así, Shamaila y col. (1996) encontraron pérdidas de volátiles superiores al 50% tras el escaldado de zanahoria en ebullición durante 60 s.

En vegetales y frutas que se someten a un escaldado previo a su deshidratación, la vitamina C se pierde en gran medida por lixiviado y oxidación a temperaturas elevadas, siendo la vitamina que se pierde con mayor facilidad durante el procesamiento y almacenamiento de los alimentos. Las variables que afectan a su degradación son el pH, la temperatura, la luz, la presencia de enzimas, oxígeno y/o catalizadores metálicos. La vitamina C resulta un indicador efectivo del impacto del procesamiento en su calidad, ya que si se retiene en gran medida es muy probable que el resto de nutrientes también sea preservado (Santos y Silva, 2008). La oxidación del ácido ascórbico puede conducir a la formación de ácido dehidroascórbico, también bioactivo, pero que se degrada irreversiblemente a ácido 2,3-dicetogulónico (Keshino y Ketitu, 1979; Ali y Sakr, 1982). Además, el ácido dehidroascórbico reacciona con aminoácidos o proteínas y puede provocar reducciones importantes en el contenido de vitamina C de alimentos deshidratados durante el procesado y especialmente durante el almacenamiento, ya que pueden originarse pigmentos pardos vía degradación de Streker (Davidek y col., 1990).

Para evitar este problema y prolongar el período de vida útil del vegetal la utilización de sulfitos en el escaldado constituye una buena alternativa (Negi y Roy, 2001). Santos y Silva (2008), en una revisión sobre la influencia del procesamiento en la retención de la vitamina C, recogieron numerosos trabajos en los que se ponía de manifiesto la importancia de los pre-tratamientos a la hora de evitar grandes pérdidas de dicha vitamina en frutas

y vegetales deshidratados. Frías y col. (2010a) escaldaron zanahoria a ebullición durante 1 min y observaron valores de retención de vitamina C próximos al 80%. Recientemente, Zhang y col. (2011) encontraron que escaldados de zanahoria a 90 °C durante 6 min provocaban una retención de vitamina C del 62,7%.

Los polifenoles o compuestos fenólicos son otros compuestos bioactivos de gran importancia en vegetales y frutas que pueden sufrir alteraciones durante su procesamiento dado que su pérdida representa una disminución considerable en la actividad antioxidante (Gorinstein y col., 2008). En la bibliografía existen estudios que muestran reducciones significativas en el contenido de compuestos fenólicos de zanahorias escaldadas en baño de agua (70-90 ºC, 1,4-25 min; Gonçalves y col., 2010). El tratamiento seleccionado por los autores como óptimo fue el de 80 ºC, durante 6 min, que provocó una retención de polifenoles totales del 70%. Estos autores observaron que las pérdidas se ocasionaban como resultado de degradaciones térmicas (autooxidación) y por lixiviado en el agua de escaldado.

1.2.2. Emergentes. Aplicación de ultrasonidos de potencia en los pre-tratamientos

Existen diversas tecnologías emergentes que se han utilizado tratamientos convencionales para escaldar vegetales y frutas. Entre ellas pueden mencionarse la aplicación de microondas (MW), el calentamiento óhmico *(*Lemmens y col., 2009), los pulsos eléctricos (Gachovska y col., 2003), la radiación infrarroja (RIR) (Krishnamurthy y col., 2008; Zhu y Pan, 2009) y las altas presiones (Yucel y col., 2010). En general, se obtuvieron resultados prometedores con todas ellos, especialmente en lo que se refiere a la reducción en el tiempo de secado posterior, respecto a los procedimientos tradicionales. El calentamiento óhmico se puede utilizar industrialmente y la RIR constituye una alternativa de interés, no sólo por la inactivación enzimática ocasionada sino también por su eficiencia energética.

Entre las tecnologías emergentes empleadas para los tratamientos previos a los procesos de secado, la aplicación de los ultrasonidos (US) de potencia ha suscitado también un gran interés (Mothibe y col., 2011).

En medio líquido, los efectos mecánicos y químicos de la cavitación parecen ser los responsables de la inactivación enzimática de los US (Raviyan y col. 2005; Tiwari y Mason, 2012). La propagación de los US tiene su fundamento en las ondas de compresión-descompresión que se inducen en las moléculas del medio a través del cual viaja la onda acústica. A partir de un determinado nivel de potencia, la descompresión da lugar a la formación de burbujas de aire de gran tamaño que, después de varios ciclos alternados de compresión-descompresión, colapsan e implosionan, liberando la energía acumulada en forma de ondas y desencadenando microcorrientes de gran velocidad capaces de alterar las características del medio (**Figura 1.1**). Estos cambios de presión y turbulencia, junto con el aumento de la temperatura en el sistema, debido a la conversión parcial de la energía acústica en calor, promueven una variedad de efectos en la matriz sonicada, como la formación de radicales libres, producto de la descomposición electroquímica de las moléculas de agua, que favorecen la inactivación enzimática (Soria y Villamiel, 2010; Fernandes y col., 2011).

Sala y col. (1995) observaron que el colapso de las burbujas de cavitación está acompañado de un aumento puntual de la presión (50 MPa) y la temperatura (5000 ºK). Estas condiciones pueden ocasionar la ruptura de puentes de hidrógeno e interacciones de Van der Waals en las cadenas peptídicas de las proteínas, con la consiguiente modificación de la estructura secundaria y terciaria (Zhong y col., 2004). Asimismo, la elevada presión y temperatura que se alcanzan favorecen la formación de radicales hidroxilos, que pueden reaccionar con residuos aminoacídicos provocando cambios en la actividad biológica de las enzimas (Barteri y col., 2004). A pesar de estos estudios, aún no se conoce con exactitud el alcance de los distintos mecanismos implicados en la inactivación y, en ocasiones, puede originarse el fenómeno opuesto de reactivación enzimática (O'Donell y col., 2010).

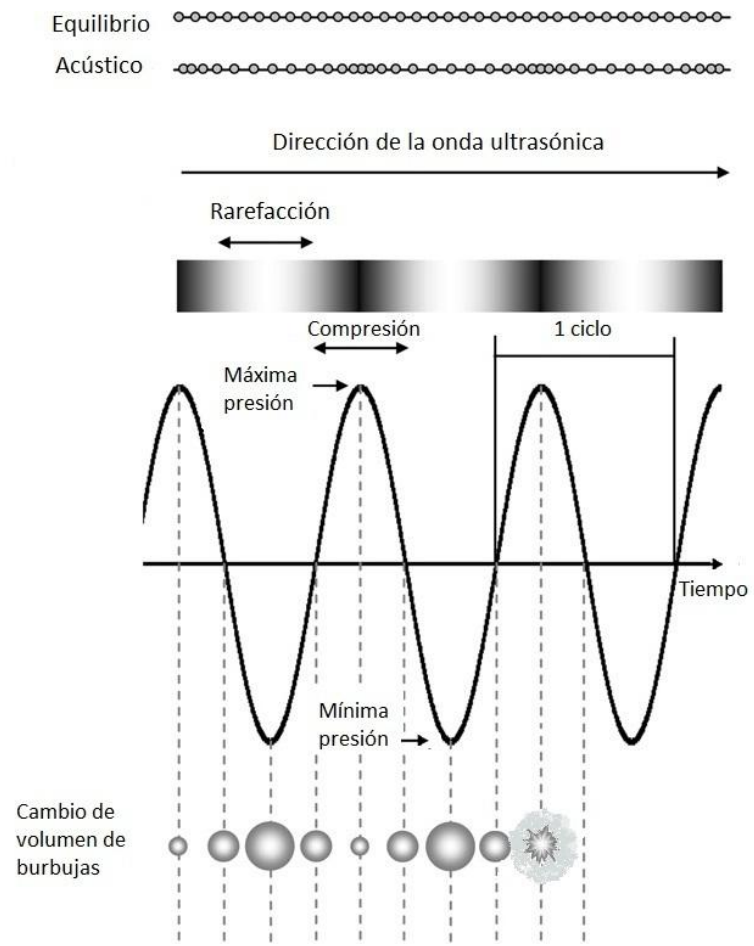

Figura 1.1 Representación esquemática del fenómeno de cavitación e implosión de burbujas de aire.

Terefe y col. (2009) encontraron un efecto sinérgico entre la temperatura y los US en sus estudios sobre la cinética de inactivación de poligalacturonasa y PME en zumo de tomate. Cruz y col. (2006), en un estudio cinético realizado en berros, demostraron el mismo efecto de la termosonicación sobre la inactivación de la POD a temperaturas entre 85 y 92,5 ºC.

Jambrak y col. (2007a) observaron reducciones en los tiempos de procesado y mejora de las propiedades de rehidratación en champiñones, coles de Bruselas y coliflor, sometidos a un proceso de secado convectivo y liofilización, que habían sido pre-tratadas con US (sonda, 20 kHz, y baño, 40 kHz, durante 3 y 10 min). Los mismos autores, (Jambrak y col., 2007b) analizaron el efecto de los US en el pH, la conductividad eléctrica y la textura de los tejidos vegetales antes estudiados. Como resultado del tratamiento con sonda de US, respecto al control sin US, observaron una disminución en

el pH del agua de escaldado, un aumento de la conductividad eléctrica por pérdida de electrolitos y modificaciones en la textura de los vegetales.

La efectividad del pre-tratamiento con US sobre la inactivación microbiana también ha sido recientemente estudiada en berros. Los tratamientos de US a 65 ºC, provocaron un mayor impacto sobre la reducción de coliformes que un tratamiento convencional llevado a cabo a la misma temperatura (Alexandre y col., 2011).

Muy recientemente, Aday y col. (2013) han empleado los US (30, 60, 90 W) como método para alargar la vida útil de fresa fresca. Dichos autores indicaron que, de acuerdo a los parámetros estudiados (composición de gas en el envase y pH), los US a potencias de 30 y 60 W prolongaban hasta 4 semanas la vida útil de la fresa sin detrimento de su calidad, en comparación con muestras tratadas con agua destilada pero sin US que se deterioraban en la primera semana.

Respecto a cambios en la composición durante el escaldado de vegetales, Rawson y col. (2011) encontraron una mayor retención de carotenoides y poliacetilenos en zanahorias deshidratadas por convección previamente tratadas de modo intermitente durante 3-10 min con US (0,39-0,95 W/mL), que en las muestras que antes del secado habían sido escaldadas a 80 °C durante 3 min.

La DO es otro claro ejemplo de la aplicación de US en medios líquidos. Como se ha indicado con anterioridad, en el caso de las frutas, suele ser habitual emplear este pre-tratamiento antes del secado convectivo, utilizando diferentes soluciones de azúcares a concentraciones, temperaturas y tiempos variables. Los efectos mecánicos de los US, que conllevan la formación de microcanales en la estructura del producto, junto con la presión osmótica son los responsables, en primer lugar, de acelerar la pérdida de agua y ganancia de sólidos y, en segundo lugar, de la reducción del tiempo de secado posterior. Esta tecnología se ha utilizado con éxito en diversos productos, tales como plátano (Fernandes y col., 2007), piña (Fernandes y col., 2008a), papaya (Fernandes y col., 2008b), fresa (García-Noguera y col., 2010) y manzana (Oliveira y col., 2011). En relación a la pérdida de sólidos, se ha observado que frutas con elevados porcentajes de humedad inicial suelen perder más sólidos que aquéllas con porcentajes inferiores, lo cual tiene su explicación en el arrastre de partículas sólidas con el agua intrínseca del

alimento. Esta pérdida también se ha visto influenciada por el efecto de los US en la estructura del tejido de la fruta, siendo el melón, la papaya y la piña especialmente sensibles a la formación de canales microscópicos en su estructura (Fernandes y col., 2008a; 2008b; 2009; Rodrigues y col., 2009a).

1.3. Procesos de deshidratación de vegetales y frutas

La deshidratación de alimentos puede llevarse a cabo por diferentes métodos. En sus inicios el secado al sol era el procedimiento más utilizado y, en la actualidad, sigue empleándose en países cálidos y secos. Aunque resulta una técnica muy barata y sencilla, la velocidad de deshidratación es muy lenta y la calidad de los productos finales es inferior a la de otros productos deshidratados. Con frecuencia, los productos secados al sol, presentan contaminaciones por insectos y partículas de suciedad (Doymaz, 2004a). En la industria, los métodos por aire caliente o convección son los preferidos (Singh y col., 2012). En el secado por convección se deshidrata, en parte, por efecto de temperaturas relativamente elevadas.

Otro de los procedimientos que ha sido estudiado profusamente a lo largo de las últimas décadas es la liofilización, que requiere una mención aparte por las características del proceso y del producto resultante.

1.3.1. Convencionales

1.3.1.1. Liofilización

La liofilización consiste en la eliminación del agua de un producto por sublimación y desorción. El hielo se transforma directamente en vapor cuando la presión de vapor y la temperatura se encuentran por debajo del punto triple (4,5 mm Hg y 0 ºC) (Alzamora y col., 2008). La deshidratación es más rápida durante la sublimación, cuando hay disponible una gran cantidad de agua no ligada en estado congelado. Durante la desorción, la velocidad de deshidratación es mucho más lenta por la dificultad de extraer el agua ligada que se encuentra en estado líquido (Vega-Mercado y col., 2001).

En la **Figura 1.2** se expone una representación esquemática de un liofilizador clásico.

Dado que el alimento se deshidrata desde el estado congelado el deterioro es escaso y, en consecuencia, resulta un producto de excelente calidad (Santos y Silva, 2008; Huang y col., 2012). Los productos liofilizados se caracterizan por una mayor rigidez estructural, elevada capacidad de rehidratación y baja densidad, a la vez que conservan la apariencia, sabor y aroma de los productos de partida (Alzamora y col., 2008). Por estas razones, la liofilización sigue siendo el método de referencia. Sin embargo, este proceso se reserva en la industria para alimentos de un alto valor añadido, ya que el coste del mismo (inversión inicial, mantenimiento de equipos y consumo energético) supera en gran medida a los gastos generados por los procesos clásicos de deshidratación (Hernando y col., 2008; Huang y col., 2012).

Gran parte de las investigaciones en liofilización se han enfocado a reducir los tiempos de procesamiento y disminuir el consumo de energía, controlando la intensidad de calor y la presión de vacío empleada. Para lograr el objetivo de acelerar el proceso, una de las propuestas ha sido operar a presión atmosférica utilizando aire frío (Ratti, 2008).

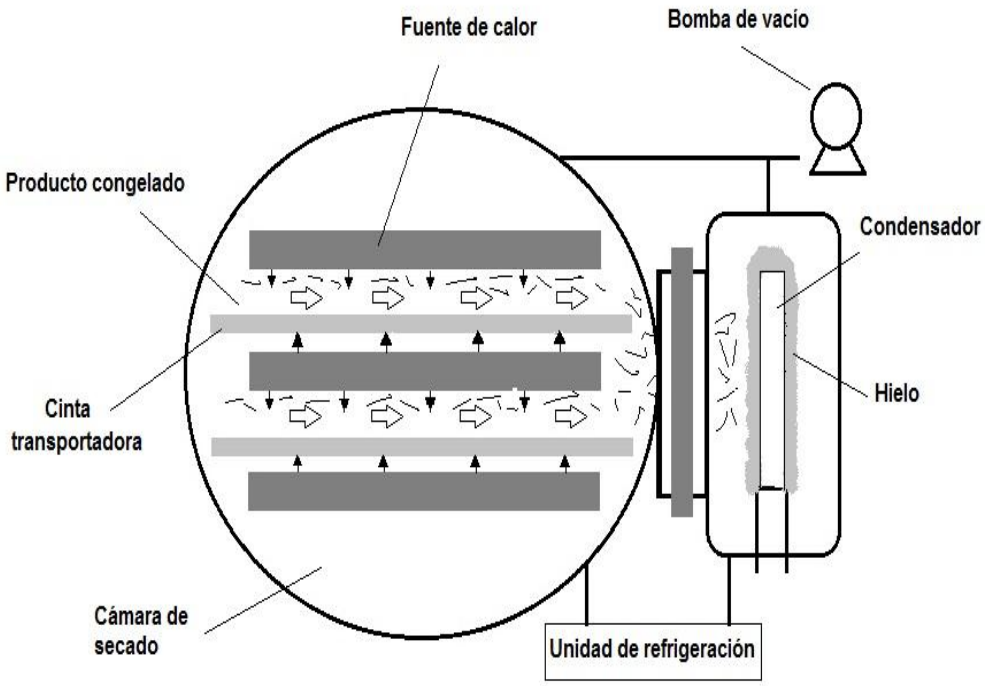

Figura 1.2 Representación esquemática de un liofilizador (tomado de Alzamora y col., 2008).

1.3.1.2. Secado convectivo

Dadas las desventajas del secado solar y los elevados costes de la liofilización, el secado convectivo es el procedimiento más empleado a nivel industrial. En la actualidad, un sinnúmero de hortalizas y frutas se deshidratan por este método, siendo manzana, tomate, ciruela, patata, zanahoria y cebolla claros ejemplos (Sagar y Kumar, 2010).

De modo general, se utilizan equipos de secado por convección forzada con aire caliente o a vacío (**Figura 1.3**). En la convección forzada, el aire caliente proporciona la fuente de calor necesaria para evaporar el agua, mientras que en los sistemas a vacío se baja el punto de ebullición del agua con la aplicación de diferentes grados de presión. El sistema a vacío, si bien da buenos resultados en lo que se refiere al aumento en la velocidad de secado, conlleva un coste elevado (Mitra y col., 2011).

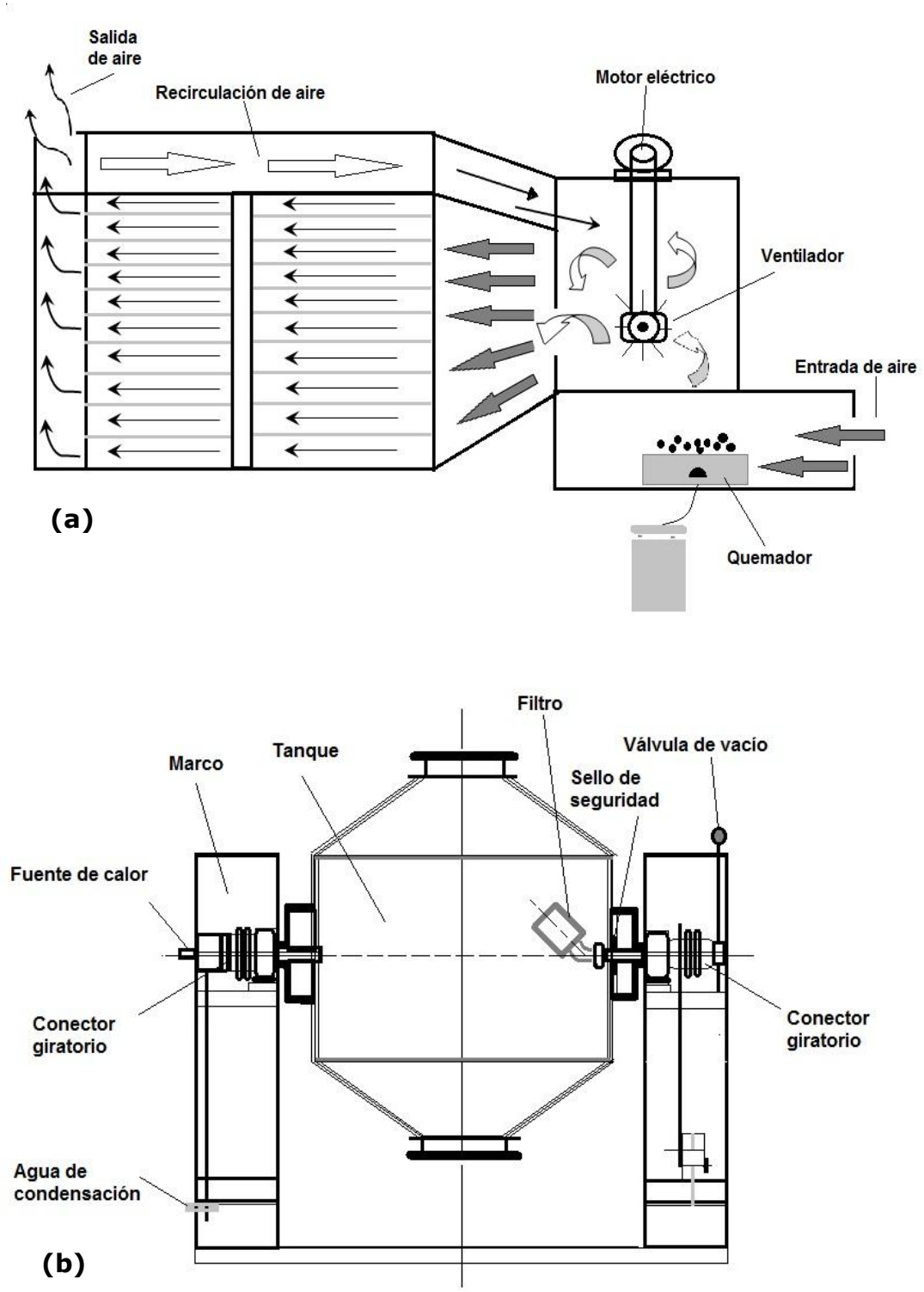

Figura 1.3 Esquemas de un equipo de secado por convección de bandejas (a) y de un sistema de deshidratación mediante vacío (b) (adaptado de Madamba, 2008 y www.drying-equipment.com).

En los equipos por convección con aire caliente, las condiciones operativas que se utilizan para deshidratar vegetales son muy variables; las temperaturas suelen oscilar entre 40 y 80 ºC, las velocidades de aire entre

0,5 y 10 m/s y los tiempos pueden prolongarse hasta 20 h (Doymaz, 2004b). La duración del proceso depende del tipo, contenido inicial de humedad y geometría del producto, así como de la temperatura y velocidad de aire aplicada durante el proceso, sin olvidar la humedad relativa ambiente (Potter y Hotchkiss, 1995).

Entre los diferentes trabajos sobre el secado de hortalizas y frutas llevados a cabo, algunos se ellos se han centrado en el estudio de los parámetros que inciden en la duración del proceso (Devahastin y Niamnuy, 2010). En estos estudios se persigue disminuir los costes energéticos, ya que el secado por convección con aire caliente requiere gran consumo de energía. La legislación sobre contaminación, tecnologías sostenibles y medio ambiente, han hecho hincapié en la necesidad de procesos energéticamente eficientes, en los que se minimicen las pérdidas, se maximice el aprovechamiento energético y se evite el sobre-procesamiento del producto (Rahman, 2003). En este sentido, Aghbashlo y col. (2009) estudiaron la deshidratación de zanahoria en un equipo semi-industrial de secado en continuo, en el rango de temperaturas 50-70 ºC, obteniéndose una mayor eficiencia energética en comparación con datos de referencias previas de secado en bandejas.

Uno de los aspectos más relevantes para poder optimizar las condiciones del proceso y así disponer de procedimientos más eficientes es conocer la cinética de la pérdida de humedad. Durante el secado la velocidad de eliminación de agua disminuye, independientemente de las condiciones establecidas en el proceso. Al comienzo de la deshidratación, y durante cierto tiempo, se puede considerar que el agua se evapora a una velocidad constante, como si se eliminase de una superficie libre. Esta etapa se denomina "período de velocidad constante". A continuación de dicha fase se produce una inflexión en la curva de secado que conduce al "período de velocidad decreciente" (**Figura 1.4**).

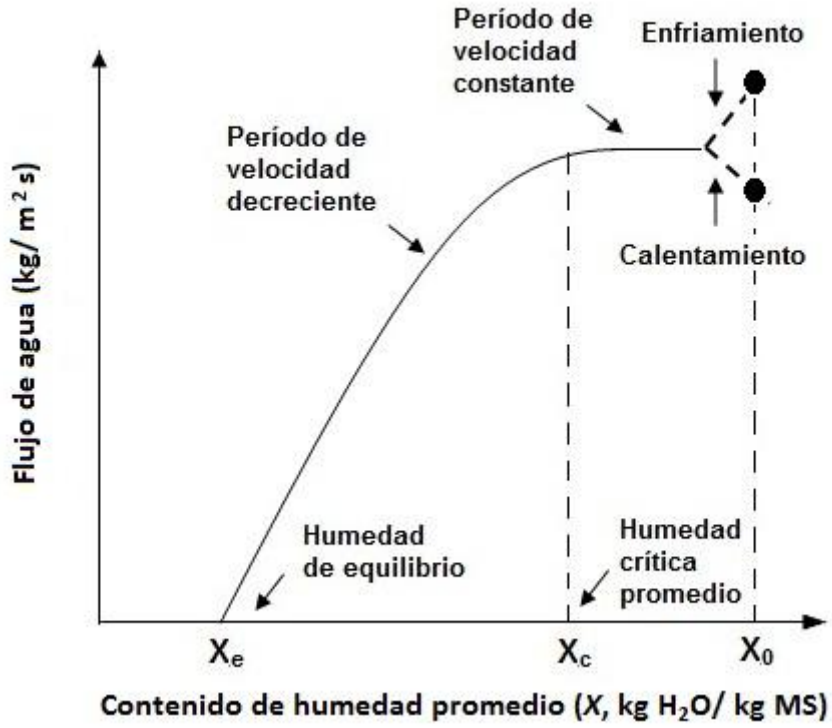

Figura 1.4 Curva de secado típica (adaptado de Chen, 2008).

Los cambios en la velocidad de secado que ocurren durante la deshidratación pueden explicarse mediante los fenómenos de transferencia de calor y de materia, como se explica a continuación.

1.3.1.2.1. Transferencia de calor y de materia

Durante el secado, el alimento pierde humedad desde su superficie, debido al calentamiento. A medida que la deshidratación evoluciona, el alimento desarrolla una costra cada vez más gruesa en la superficie que aísla al producto del exterior y retrasa la trasferencia de calor hacia el interior (Farid, 2008). El agua retenida en el centro deberá atravesar una mayor resistencia interna para salir del alimento. Sumado a estos factores, el producto se va aproximando a la humedad relativa de equilibrio, lo que significa que absorbe moléculas de agua de la atmósfera al mismo tiempo que las pierde. El fin del proceso de secado ocurre cuando la humedad del producto alcanza dicha humedad de equilibrio (Potter y Hotchkiss, 1999).

En un proceso convectivo tradicional, los fenómenos de transporte (materia y energía) entre la superficie del sólido y el aire de secado dependen

de ciertas propiedades del aire tales como la temperatura, la humedad relativa y la velocidad. Por otra parte, los fenómenos de transporte en el interior del sólido dependen de la naturaleza del producto, su humedad y la temperatura. Así, la resistencia global a la transferencia de materia y energía se puede desglosar en dos, la localizada en la capa de aire que rodea a la superficie del sólido (resistencia externa, RE) y la inherente al sólido (resistencia interna, RI).

Durante el secado de alimentos, el mecanismo de difusión es considerado como responsable de la transferencia de materia desde el interior del sólido hacia la superficie. Así, la transferencia de agua es descrita por la difusividad efectiva de materia (D_e). Para describir el fenómeno de difusión de agua dentro del sólido, durante el proceso de secado, se ha utilizado la sunda Ley de Fick (ecuación 1) (Simal y col., 2003).

$$\frac{\partial W_p(x,t)}{\partial t} = D_e \frac{\partial^2 W_p(x,t)}{\partial x^2} \qquad (1)$$

donde W_p es el contenido de humedad local de la muestra (kg H_2O/kg MS), D_e es la difusividad efectiva (m^2/s), t es el tiempo (s) y x (m) representa la dirección de transporte característica para la geometría de lámina. La D_e es el parámetro cinético que representa la facilidad del agua para difundir hacia la superficie del sólido durante el proceso de secado.

Los supuestos más generales utilizados para el análisis difusional son: (i) el producto es unidimensional y tiene un contenido de humedad inicial uniforme, (ii) la transferencia de agua se produce únicamente desde el interior del sólido hacia la superficie (se considera la resistencia interna al flujo de humedad como la resistencia dominante), (iii) el sólido no presenta encogimiento ni deformación durante el secado y (iv) es despreciable el efecto de la transferencia de calor interno y externo. En la resolución de la ecuación (1), mediante el modelo que considera despreciable la resistencia externa a la transferencia de materia (*SRE*), se utilizan la condición inicial (2) y las condiciones de contorno expuestas en las ecuaciones (3) y (4).

$$t = 0 \qquad W_p(x,0) = W_0 \tag{2}$$

$$\begin{matrix} t > 0 \\ x = 0 \end{matrix} \qquad \frac{\partial W_p(0,t)}{\partial x} = 0 \tag{3}$$

$$\begin{matrix} t > 0 \\ x = L \end{matrix} \qquad W_p(L,t) = W_e \tag{4}$$

donde W_0 representa el contenido de humedad inicial de la muestra (kg H_2O/kg MS), L el semiespesor (m) y W_e la humedad de equilibrio (kg H_2O/kg MS).

La solución a la ecuación de Fick para diferentes geometrías fue propuesta por Crank (1975). En particular, la solución para la geometría de lámina infinita del modelo *SRE*, en términos de contenido de humedad media, se presenta en la ecuación (5).

$$W_p(t) = W_e + (W_c - W_e) \times \left[\sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left(- \frac{D_e (2n+1)^2 \pi^2 t}{4L^2} \right) \right] \tag{5}$$

donde W_c es el contenido de humedad crítica (kg H_2O/kg MS).

La identificación de la D_e en procesos de deshidratación según el modelo *SRE* ha sido objeto de numerosos estudios (Doymaz, 2004b; Simal y col., 2005). En general, los valores de D_e para matrices alimentarias publicados se encuentran entre 10^{-11} y 10^{-9} m^2/s (Doymaz, 2008b).

Con el objetivo de evaluar la influencia de la resistencia externa en las cinéticas de secado (Bon y col., 2007; Giner, 2009), se ha propuesto el modelo RE. En este caso, para resolver la ecuación (1) se ha utilizado una condición de contorno que considera el flujo de agua entre la superficie del sólido y del aire (ecuación 6).

$$\begin{matrix} t > 0 \\ x = L \end{matrix} \qquad - D_e \rho_{ds} \frac{\partial W_p(L,t)}{\partial x} = k(a_w(L,t) - \varphi_{air}) \tag{6}$$

donde, p_{ds} es la densidad del sólido seco (kg MS/m^3), k es el coeficiente convectivo de transferencia de materia (kg H_2O/m^2/s), a_w es la actividad de agua en la superficie del sólido y φ_{air} es la humedad relativa del aire de secado.

Por último, otra de las modificaciones que mejoran el ajuste del modelo es considerar que el volumen del sólido varía durante el proceso de secado debido al encogimiento del producto (Simal y col., 2005; Aversa y col., 2011; Brasiello y col., 2013). El encogimiento volumétrico se ha correlacionado con el contenido de humedad aplicando funciones lineales y no lineales del tipo de las mostradas en las ecuaciones 7 y 8 (Mayor y Sereno, 2004; Devahastin y Niamnuy, 2010).

$$\frac{V}{V_0} = f(X) \tag{7}$$

$$\frac{V}{V_0} = f\left(\frac{X}{X_0}\right) \tag{8}$$

donde V_0 y V son el volumen del material inicial y el volumen a tiempo "t" durante la deshidratación; X_0 y X son los contenidos de humedad inicial y a tiempo "t".

Por todo ello, es preciso conocer las características de la matriz a deshidratar y del propio proceso para poder explicar el mecanismo de transferencia de materia que tiene lugar durante el secado. Así, la complejidad del modelo difusional que se elija dependerá del grado de conocimiento que se persiga con el fin último de optimizar el proceso.

1.3.1.3. Modificaciones en los constituyentes

Dadas las características del secado convectivo, junto con la necesaria eliminación de agua, se producen una serie de modificaciones en los constituyentes de vegetales y frutas que alteran la calidad global de los mismos y provocan pérdidas importantes en los compuestos bioactivos. A continuación se ofrece una revisión sobre algunos de los cambios más importantes que tienen lugar.

1.3.1.3.1. Reacción de Maillard

El pardeamiento no enzimático engloba una serie de reacciones químicas entre las que se encuentra la reacción de Maillard (RM) y la caramelización. Por las condiciones de temperatura y a_w que se alcanzan durante la deshidratación, la RM se ve favorecida frente a la caramelización (Ramírez-Jiménez y col., 2001). La RM se produce entre un grupo amino libre de un aminoácido, péptido o proteína y el grupo carbonilo de un azúcar reductor, tales como glucosa o fructosa en vegetales y frutas. El avance de la reacción depende de la temperatura, la a_w, el pH, la concentración de oxígeno, la naturaleza y la concentración de carbohidratos y proteínas (Olano y Martínez-Castro, 1996).

La RM puede subdividirse en tres etapas diferenciadas, en cada una de ellas tienen lugar múltiples reacciones que involucran la formación de diversos compuestos. En las etapas iniciales de la reacción se originan los compuestos de Amadori (1-amino-1-desoxi-2-cetosa) (**Figura 1.5**), primeros productos estables de la misma (Finot y Mauron, 1972; O'Brien y Morrissey, 1989; Nuñez y Laencina, 1990), y los compuestos de Heyns (2-amino-2-desoxialdosas) (Nursten, 1981; Matsuda y col., 1991).

Posteriormente, la reacción progresa hacia las etapas avanzadas donde se forman compuestos dicarbonilos y productos avanzados de la glicación. Las etapas finales se caracterizan por la formación de compuestos coloreados de tipo polimérico denominados melanoidinas.

Gran parte de los trabajos que se recogen en la bibliografía referidos al avance de la RM en vegetales deshidratados se han centrado en el estudio del pardeamiento que se origina a consecuencia de estados avanzados de la reacción (Krokida y col., 2001; Negi y Roy, 2001; Kim y col., 2004; Kaymak-Ertekin y Gedik, 2005).

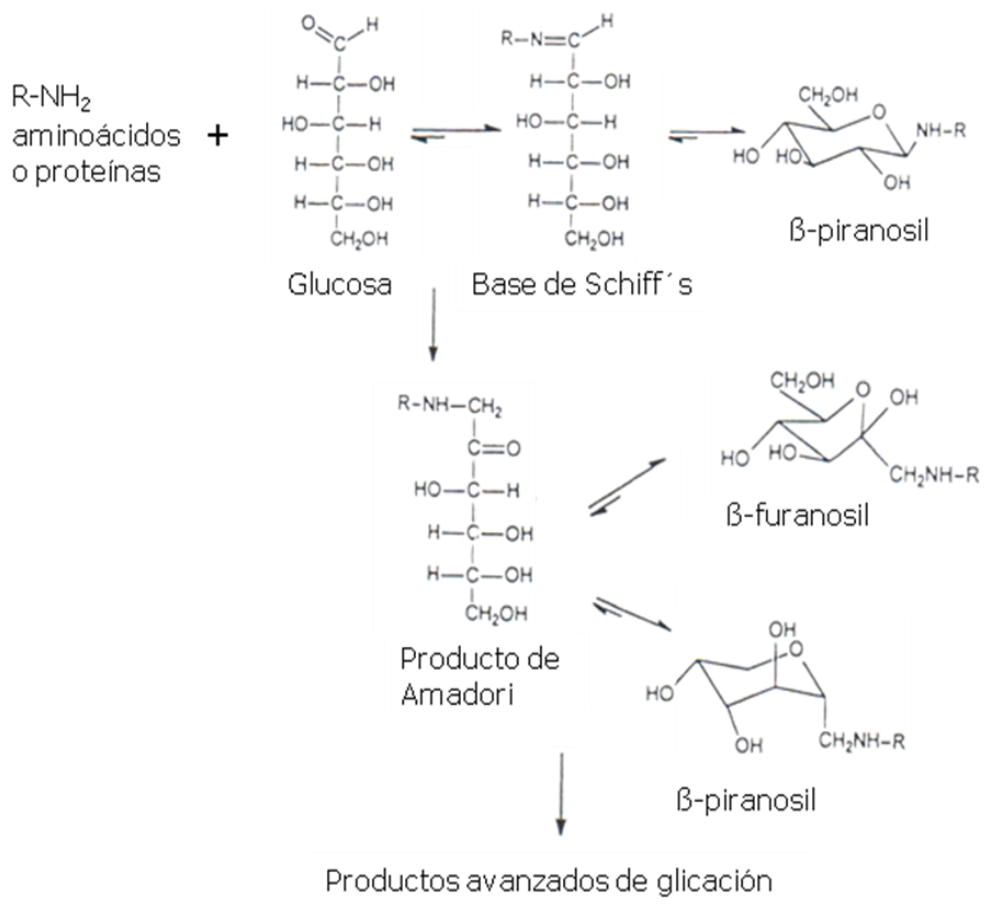

Figura 1.5 Etapas iniciales de la reacción de Maillard (modificado de Friedman, 2003)

Otros trabajos se han enfocado a la determinación de compuestos formados en etapas avanzadas de la RM, como el HMF, originado también por deshidratación de las hexosas. El HMF es un reconocido indicador de deterioro de la calidad de alimentos ricos en carbohidratos y que han sido sometidos a un excesivo calentamiento o almacenamiento inadecuado (Olano y Martínez-Castro, 1996). Aunque el HMF se ha utilizado en frutas (Fernández-Artigas y col., 1999) y en vegetales deshidratados (Soria y col., 2009b), Rufián-Henares y col. (2008) lo detectaron únicamente en ajo, cebolla y tomate, de un total de 42 muestras comerciales de vegetales deshidratados. Asimismo, a pesar de ser un indicador de uso habitual en la industria alimentaria, la eficiencia del HMF como parámetro de calidad ha sido cuestionada por su dudosa capacidad de evaluar los daños a bajas temperaturas (Hidalgo y col., 1998).

Paralelamente se ha estudiado otro indicador, la furosina (ε-N-(2-furoilmetil-L-lisina) obtenida por la hidrólisis ácida del compuesto de Amadori

derivado de la lisina, formado durante las etapas iniciales de la RM. La utilidad de la furosina como indicador de la intensidad de los tratamientos térmicos se ha reflejado en numerosos estudios realizados sobre alimentos de origen vegetal (Resmini y Pellegrino, 1991; Hidalgo y col., 1998; Guerra-Hernández y col., 1999; Sanz y col., 2001; Rada-Mendoza y col., 2004; Rufián-Henares y col. 2008). Además de la furosina, también se han determinado otros 2-furoilmetil derivados de la alanina, arginina y ácido γ-aminobutírico en zumo de naranja (Del Castillo y Olano, 1999) y en ajos, cebollas y zanahorias deshidratadas (Cardelle-Cobas y col., 2005; Soria y col., 2009b; Wellner y col., 2011). Respecto a la cinética de la formación de los 2-furoilmetil aminoácidos (2-FM-AA) en alimentos de origen vegetal, tan sólo se ha estudiado en productos derivados del tomate, encontrándose que es de orden cero (Hidalgo y Pompei, 2000).

Los 2-FM-AA han demostrado ser eficaces en la evaluación de las etapas iniciales de la RM, proporcionando una información muy valiosa para el control de procesos, ya que permite seguir la reacción en etapas en las que aún no se han producido importantes alteraciones en el valor nutritivo y en las características organolépticas del alimento.

1.3.1.3.2. Modificaciones en vitaminas y polifenoles

La vitamina C, además de perderse durante los pre-tratamientos, puede también sufrir pérdidas durante el proceso de deshidratación. El efecto del secado por aire caliente sobre la vitamina C se ha estudiado en diferentes tipos de vegetales y frutas, tal y como se refleja en la revisión llevada a cabo por Santos y Silva (2008). En zanahoria se ha visto que el ácido ascórbico es especialmente sensible a los períodos largos de secado, mientras que los carotenoides resultan más sensibles a la temperatura (Mohamed y Hussein, 1994). Negi y Roy (2001) encontraron un 46% de retención de la vitamina C en zanahorias deshidratadas por convección a 65 °C previamente escaldadas a 95 °C durante 30 s. Frías y col. (2010a), en zanahorias escaldadas 1 min en ebullición y deshidratadas en un secador de bandejas a 40-65 °C durante 6 h, observaron valores de retención de vitamina C en el intervalo 32-50%.

En frutas, la fresa ha sido una de las más estudiadas por su elevado contenido en vitamina C. Los trabajos de Asami y col. (2003) sobre

liofilización y secado convectivo, El-Beltagy y col. (2007) de secado solar y Wojdylo y col. (2009) sobre secado por MW a vacío, abordaron el estudio de las pérdidas de ácido ascórbico en fresas deshidratadas y encontraron retenciones muy variables, entre el 16 y 95%, dependiendo del proceso de deshidratación.

La degradación de ácido ascórbico puede ajustarse a una cinética de primer orden según se ha comprobado en vegetales y frutas deshidratados tales como patata (McMinn y Magee, 1997a; Khraisheh y col., 2004), piña (Ramallo y Mascheroni, 2004), rosa mosqueta (Erenturk y col., 2005; Pirone y col., 2007), guayaba (Sanjinez-Argandoña y col., 2005), tomate (Goula y Adamopoulos, 2006) y kiwi (Orikasa y col., 2008). La temperatura, la a_w de la muestra, la humedad y la concentración de oxígeno son las principales variables estudiadas para evaluar la pérdida de dicha vitamina durante el proceso de deshidratación (Santos y Silva, 2008).

En el caso de las zanahorias deshidratadas es de particular relevancia la pérdida de ß-caroteno, precursor de la vitamina A, responsable del color (Goldman y col., 1983) y de la capacidad antioxidante (Hiranvarachat y col., 2008). Además, al igual que otros constituyentes, el ß-caroteno se vincula con ciertos beneficios para la salud, tal y como se ha indicado previamente (Baker y Günter, 2004; Demir y col., 2004). Durante los tratamientos térmicos, el ß-caroteno es capaz de disolverse parcialmente en lípidos celulares, lo que implica una elevada susceptibilidad a la degradación (Reither y col., 2003). Por otra parte, la pérdida de humedad durante el proceso de deshidratación favorece la oxidación autocatalítica del ß-caroteno (Goldman y col., 1983). Numerosos estudios se han centrado en el efecto del procesamiento y almacenamiento en el contenido de ß-caroteno, debido a su relevancia como marcador sensible de la intensidad del tratamiento térmico (Suvarnakuta y col., 2005). También durante la deshidratación pueden originarse degradaciones de los carotenoides no sólo debido a cambios químicos sino a las alteraciones físicas de los tejidos (Arya y col., 1979). El efecto adverso del secado convectivo sobre la pérdida de β-caroteno fue también estudiado por Soria y col. (2009b) en zanahorias, en este caso obtenidas industrialmente. Estos autores encontraron pérdidas de dicho

constituyente de hasta 35% tras diferentes etapas de secado realizadas a temperaturas comprendidas entre 60 y 110 °C.

Otros compuestos importantes que contribuyen a la actividad antioxidante en los vegetales son los polifenoles. En zanahorias deshidratadas por diferentes métodos (secado por convección, MW, RIR y liofilización), Witrowa y col. (2009), analizaron los contenidos de antocianinas, polifenoles y la actividad antioxidante. De sus resultados concluyeron que el secado con RIR para la variedad *Deep Purple* y la liofilización (-20 ºC) para la variedad *Purple Haze,* eran mejores técnicas que el secado convectivo para obtener contenidos elevados de compuestos bioactivos. En fresas tratadas por altas presiones y por convección, MW o vacío se vio una mayor estabilidad de los polifenoles que de vitamina C (Patras y col., 2009; Wojdylo y col., 2009).

1.3.1.3.3. Cambios en las propiedades organolépticas

Las modificaciones químicas y físicas que tienen lugar durante los pre-tratamientos y los tratamientos de deshidratación a los que son sometidos las frutas y los vegetales pueden incidir en la calidad sensorial de los mismos. En este sentido, los volátiles, los azúcares, los pigmentos y diversos compuestos bioactivos, entre otros, pueden verse afectados durante el procesado, alterando las propiedades organolépticas del producto final (Shamaila y col., 1996; Soria y col., 2008; Azeredo, 2009).

Con respecto a la textura y, como se ha indicado anteriormente, los pre-tratamientos pueden ser claves en la firmeza del producto final, debido al grado de inactivación de enzimas como la PME. Además, un excesivo pre-tratamiento puede afectar a la estructura del tejido vegetal dando lugar a una mayor capacidad de rehidratación en el producto final. No obstante, esto no implica necesariamente una mejora de la calidad, dado que la desestructuración del tejido puede originar una textura demasiado blanda, lo cual perjudicará las características organolépticas del producto y su grado de aceptación por el consumidor (Sanjuán y col., 2005; Azuara y col., 2009).

Durante el secado por convección, especialmente a altas temperaturas, la estructura vegetal se altera debido al encogimiento y a la formación de una costra en la superficie, lo que incide negativamente en la capacidad de

rehidratación del producto final y, consecuentemente, en su valoración sensorial (Cui y col., 2008).

La mayor parte de los estudios se han llevado a cabo sobre la evaluación sensorial de los productos tras su rehidratación, dado que, de acuerdo con Lin y col. (1998), el color, la apariencia, la textura, el flavor y la aceptabilidad global de zanahorias secadas por aire caliente mejora cuando son rehidratadas; además dichos productos suelen consumirse tras una fase de rehidratación. Sin embargo, es preciso tener en cuenta que también el propio proceso de rehidratación, dependiendo de las condiciones, produce cambios en la estructura y en la composición de los tejidos, modificando las propiedades del producto reconstituido. Durante la rehidratación se produce una considerable pérdida de sólidos (vitaminas, azúcares, aminoácidos y minerales) por difusión que ocurre a una velocidad mayor que la absorción de agua (García-Pascual y col., 2006).

Lin y col. (1998) no encontraron diferencias significativas en la valoración global de zanahorias previamente escaldadas a 90 °C durante 7 min y sometidas a deshidratación mediante convección, MW con vacío o liofilizadas. Marabi y col. (2006), por el contrario, observaron que muestras comerciales de zanahoria deshidratada a vacío presentaron una mayor aceptación general que las muestras deshidratadas convencionalmente. Además, los tiempos de rehidratación tuvieron una influencia significativa sobre la textura percibida por el panel. El efecto de la temperatura de secado en la calidad sensorial del producto final se ha estudiado también en banana deshidratada encontrándose una mayor aceptación del producto a las temperaturas más suaves (Leite y col., 2007).

Otro de los aspectos relacionados con la calidad sensorial de vegetales y frutas deshidratados es la evaluación del efecto del proceso en el perfil de volátiles, empleando diversas técnicas analíticas. Así, los estudios de Gögüs y col. (2007) en albaricoques y Mujic y col. (2012) en higos, se centraron en la caracterización de los volátiles mediante GC-MS tras diversos procesos de deshidratación de dichas frutas.

En zanahorias, se han utilizado diferentes técnicas de fraccionamiento y concentración, para analizar por GC-MS las muestras en función de su composición en volátiles (Shamaila y col., 1996; Soria y col., 2008). Además, se han ensayado nuevas metodologías basadas en GC-MS, denominadas

pseudonariz electrónica o Sensor Químico (ChemSensor). El sensor químico permite obtener una huella digital química correspondiente al perfil global de volátiles presente en la muestra, sin necesidad de una completa separación cromatográfica. Posteriormente, mediante la interpretación quimiométrica de los datos obtenidos, es posible clasificar las muestras de acuerdo a diferentes criterios prefijados. Esto puede resultar una herramienta excelente como complemento del análisis sensorial. La técnica de pseudonariz electrónica, se ha utilizado con éxito para clasificar aceites de oliva (Peña y col., 2002) y vinos (Dirinck y col., 2006), entre otros productos. En zanahoria, esta técnica se ha utilizado para clasificar muestras sometidas a almacenamiento (Vikram y col., 2006).

1.3.2. Emergentes

Dado que los procesos clásicos de deshidratación de vegetales y frutas presentan ciertas desventajas, se está explorando la aplicación de tecnologías emergentes que los sustituyan o que los complementen y mejoren. Todo ello encaminado a obtener productos deshidratados de una alta calidad organoléptica y nutricional y que, en la medida de lo posible, preserven las propiedades funcionales presentes naturalmente en los vegetales y frutas, empleando procesos que resulten energéticamente eficientes y respetuosos con el medio ambiente (Cárcel y col., 2012).

En la búsqueda de dichas tecnologías se ha investigado la deshidratación de vegetales y frutas mediante la aplicación de MW, como un procedimiento único o en combinación con aire caliente o vacío (Fito y Chiralt, 2003; Natella y col., 2010; Feng y col., 2012; Ghanem y col., 2012). En general, puede decirse que hay una reducción del tiempo de secado y que la calidad de los vegetales y frutas deshidratados mediante MW es superior a la que se obtiene por procesos de convección forzada con aire caliente (Sumnu y col., 2005; Ozkan y col., 2007). A pesar de las ventajas, la utilización de MW en los procesos de secado requiere un preciso control del sistema de aplicación para evitar los fenómenos de falta de uniformidad, el calentamiento puntual y excesivo del producto que puede afectar negativamente a su calidad.

La RIR ha mostrado poseer varias ventajas entre las que pueden mencionarse: reducción en el tiempo de proceso, calentamiento uniforme de las muestras, menor pérdida de calidad, equipamiento simple, compacto y versátil y un bajo consumo energético (Junling y col., 2008; Rastogi, 2012). Pese a que existen resultados alentadores, la principal limitación de la aplicación de RIR en la deshidratación es la escasa penetración de la radiación. La energía RIR es absorbida en la superficie del producto, transfiriéndose a otras áreas por conducción y, a medida que aumenta el volumen de muestra, la eficiencia del secado se reduce. Sin embargo, la combinación de esta técnica con MW u otras tecnologías, tiene un gran potencial al incrementar la transferencia de materia y obtener una distribución de humedad uniforme (Rastogi, 2012).

Además de las anteriores, entre las tecnologías emergentes que han irrumpido en los últimos años para la deshidratación de vegetales y frutas, especial interés ha despertado la aplicación de US de potencia. La principal ventaja de los US radica en su capacidad de reducir el tiempo de secado sin apenas incorporar energía térmica en el proceso, lo cual resulta especialmente beneficioso en alimentos termosensibles tales como los vegetales y las frutas (Muralidhara y col., 1985; Chemat y col., 2011; Awad y col., 2012).

1.3.2.1. Aplicación de ultrasonidos de potencia en la deshidratación de vegetales y frutas

Los procesos de deshidratación asistidos por US se clasifican en función del diseño del sistema de secado y la influencia que ejerce el campo acústico sobre la muestra, en: (i) sistemas *por contacto*, cuando la placa vibrante ultrasónica está en contacto directo con la muestra a deshidratar y (ii) sistemas *sin contacto*, cuando la muestra se dispone en una cabina aislada, que funciona como elemento vibrante, en un equipo de secado convectivo, bajo la influencia de las ondas ultrasónicas generadas por el transductor.

1.3.2.1.1. Sistemas por contacto

Cuando los US se aplican en contacto con el material que ha de ser deshidratado, las ondas se transmiten al producto sólido originando rápidas series de compresiones y expansiones que dan lugar a una migración del líquido hacia el exterior. Este fenómeno, que favorece la difusión de agua de los canales naturales o de otros creados por la propagación de las ondas, se conoce como "efecto esponja" (Gallego-Juárez y col., 1999). Además, la cavitación que originan los US en el medio líquido del interior del producto puede facilitar la pérdida de las moléculas de agua que se encuentran más fuertemente ligadas al material (Tarleton y Wakeman, 1998). Otros efectos que también pueden acelerar la cinética del proceso de deshidratación asistido con US son la presión de radiación (fuerza neta ejercida desde la fuente ultrasónica en dirección al medio) y las corrientes acústicas (vórtices formados en las proximidades de la interfase sólido-gas).

Gallego-Juárez y col. (1996) patentaron un dispositivo de deshidratación ultrasónico por contacto con un sistema de aplicación con presión estática, sistema de succión y flujo de aire auxiliar, para el tratamiento uniforme de muestras dispuestas en una placa rectangular metálica (**Figura 1.6**). Mediante este original procedimiento redujeron de forma apreciable la temperatura y el tiempo de tratamiento alcanzando pérdidas de humedad considerables, con un consumo energético reducido (Gallego-Juárez y col., 1999). Los experimentos de secado con US se llevaron a cabo en diferentes matrices vegetales como zanahorias, patatas y champiñones (De la Fuente y col., 2006; Gallego-Juárez y col., 2007).

Recientemente, Schössler y col. (2012a) investigaron la aplicación de US por contacto en cilindros de patata y estudiaron los cambios producidos en la estructura del tejido y en la transferencia de materia. De sus estudios concluyeron que el alcance de la disrupción celular, atribuida a los efectos de los US, variaba según la temperatura y la amplitud de excitación, siendo 70 ºC y 4 µm de amplitud de excitación las condiciones que afectaron en mayor medida a la transferencia de materia, incrementando la velocidad de secado. Los mismos autores (Schössler y col. 2012b) con el equipo anterior, para alcanzar una humedad residual del 20%, encontraron reducciones del tiempo de secado del 18 y del 27% en cubos de pimiento rojo y manzana,

respectivamente. Además, en manzana estudiaron la viabilidad de tratamientos intermitentes de US y encontraron que, reduciendo un 50% el tiempo neto de sonicación, apenas se modificaban los efectos de los US en la cinética de la pérdida de humedad.

También se han propuesto sistemas de US por contacto en procesos de liofilización. Así, Schössler y col. (2012c) redujeron la humedad de muestras de pimiento rojo hasta un 10% en un proceso de liofilización asistido por US con contacto y se observó una reducción en el tiempo de procesado de un 11,5% respecto a un proceso de liofilización clásico. Además, no se vio alterada la calidad del vegetal en cuanto a contenido en ácido ascórbico, densidad, color y propiedades de rehidratación.

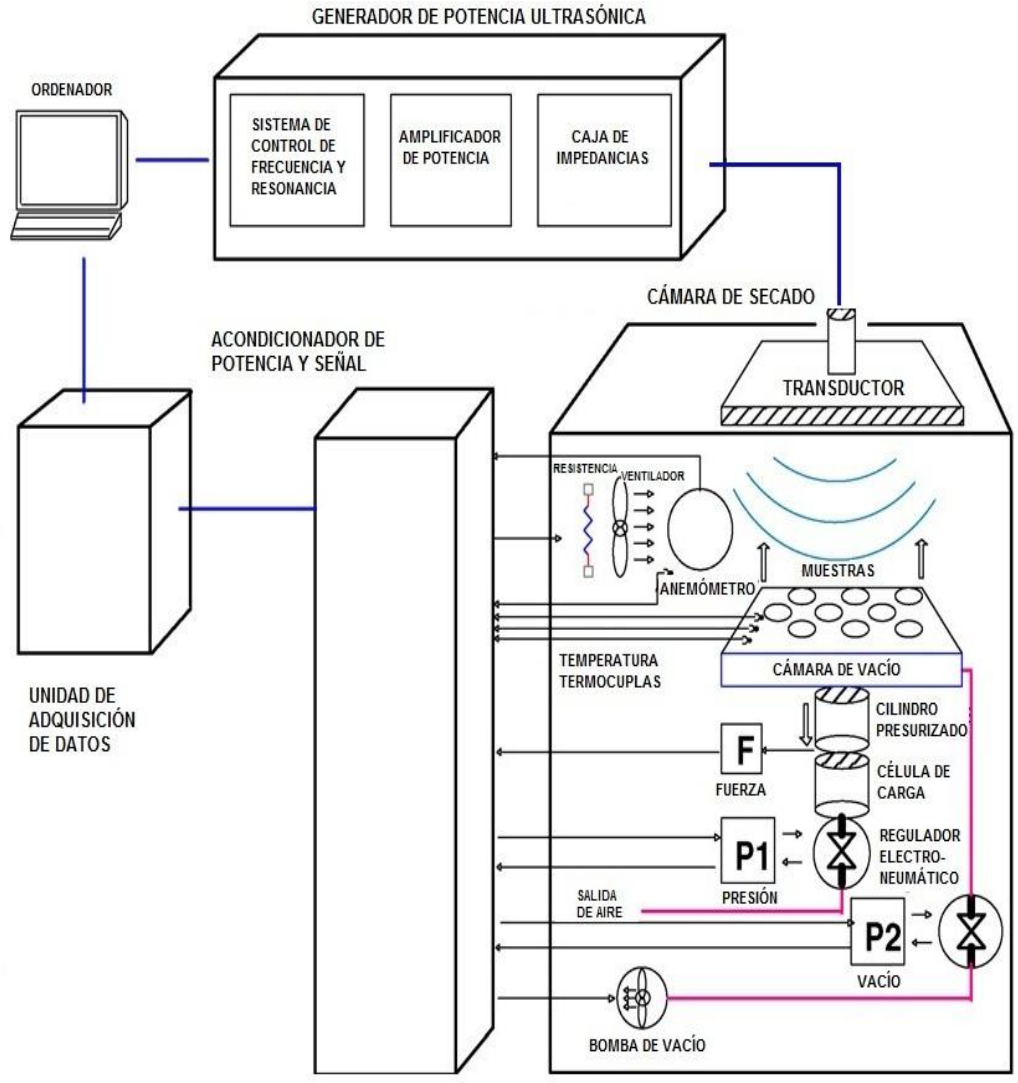

Figura 1.6 Representación esquemática del dispositivo de deshidratación asistido por US *de contacto*.

A pesar de que los estudios realizados con el prototipo de contacto mostraron resultados prometedores, el hecho de que deba mantenerse el contacto directo entre la placa vibrante y las muestras dificulta su escalado a nivel industrial. Como alternativa, García-Pérez y col. (2006a, 2007) propusieron la utilización de una cabina de secado para transmitir las ondas de US a las muestras sometidas a un secado convectivo. En este caso, se trata de un sistema de secado asistido por US de potencia, tal y como se expone a continuación.

1.3.2.1.2. Sistemas sin contacto

En los sistemas sin contacto, en una cabina de secado, un transductor ultrasónico excita un sistema de aplicación que al vibrar transmite las ondas ultrasónicas al aire para que finalmente alcancen a las partículas del producto (**Figura 1.7**). De esta forma, el material a deshidratar, sin contactar directamente con el sistema de aplicación, se mantiene bajo la influencia del campo acústico generado en la cámara de secado. En este caso, la aplicación de US se ve dificultada por la baja impedancia acústica del aire, lo que da lugar a mayores pérdidas energéticas por atenuación desde los sistemas de aplicación ultrasónicos (Cárcel y col., 2012). No obstante, y pese a las dificultades, en los últimos años se han producido avances en el diseño de los sistemas de aplicación ultrasónicos que permiten una mejor transferencia de la energía acústica hacia el aire y hacia el producto a deshidratar (García-Pérez y col., 2006b; Gallego-Juárez y col., 2010).

La aplicación de este sistema de secado asistido por US se ha investigado en diversos productos vegetales, tales como zanahorias y piel de limón (García-Pérez y col., 2009), piel de naranja (Ortuño y col., 2010), hojas de olivo (Cárcel y col., 2010) y patatas (Ozuna y col., 2011). En estos trabajos, se ha estudiado el efecto de diferentes variables del proceso como la velocidad de aire, la temperatura y la densidad de carga sobre las cinéticas de secado y las propiedades físicas de los productos resultantes.

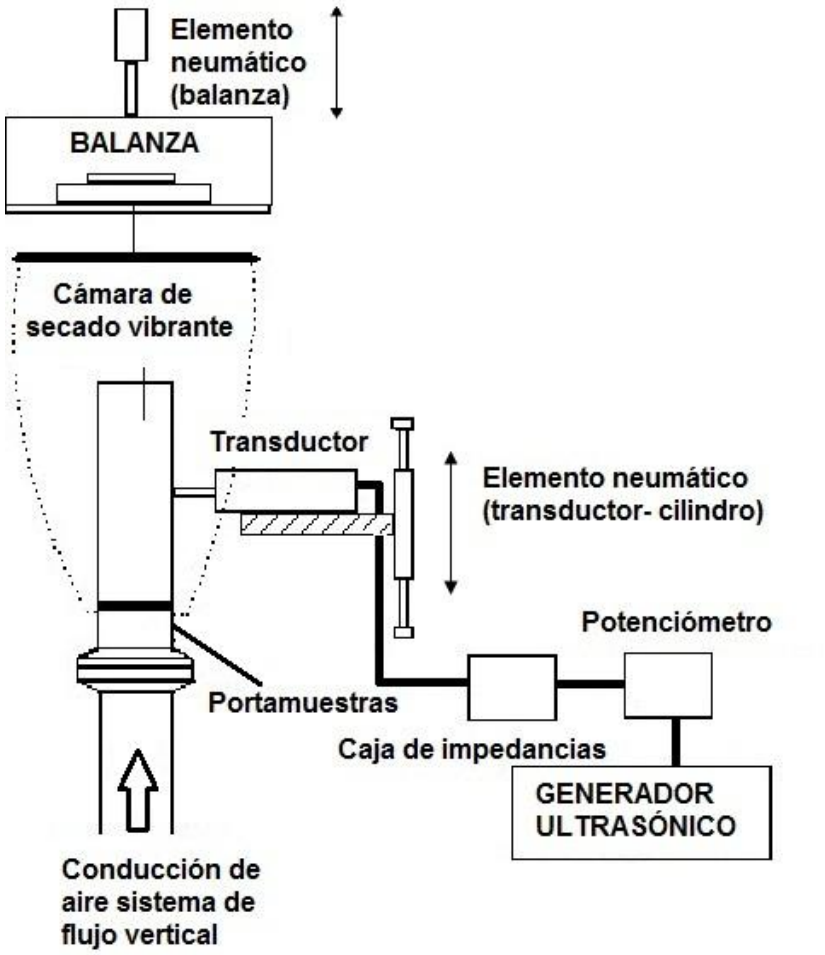

Figura 1.7 Representación esquemática del equipo de secado convectivo asistido por US *sin contacto*. (Adaptado de Cárcel y col., 2011).

En estos estudios, se ha comprobado el efecto de los US en la D_e a bajas velocidades de aire (inferiores a 2 m/s), ya que a velocidades de aire superiores, el campo acústico generado en la cabina de secado es distorsionado por el flujo de aire, reduciendo su eficacia. Este efecto se traduce en un aumento de la D_e que provoca una reducción del tiempo de secado. Este comportamiento fue estudiado por García-Pérez y col. (2006a) a partir de mediciones con un sensor de presión ubicado en la cabina de secado del equipo asistido por US. Observaron que la presión acústica disminuía a medida que aumentaba la velocidad de aire, hasta 8 m/s, velocidad a partir de la cual no se apreciaba efecto de los US. En otro estudio comprobaron que un aumento de la temperatura de 30 a 60 ºC producía una reducción del efecto de los US sobre la cinética de secado de cubos de

zanahoria deshidratados a una velocidad de aire de 1 m/s (García-Pérez y col., 2006b).

Con respecto a la potencia ultrasónica aplicada, es necesario un determinado nivel para evidenciar el efecto de los US sobre los parámetros cinéticos del proceso de secado, nivel que varía con el producto a deshidratar (Cárcel y col., 2012). En cubos de zanahoria, 25 kW/m^3 dieron lugar a diferencias apreciables en la D_e, comparado con el tratamiento control sin US. En piel de limón, el umbral de potencia para obtener diferencias en la D_e fue inferior (12 kW/m^3). Esto se explica por la elevada porosidad de la piel de limón comparada con otros sustratos vegetales, como la zanahoria. En el caso de patatas deshidratadas a potencias ultrasónicas elevadas (37 kW/m^3), se observaron reducciones de un 40% en el tiempo de secado respecto de experiencias sin aplicación de US (Ozuna y col., 2011). En piel de naranja, en un estudio reciente, se obtuvieron incrementos significativos de 47 y 108% en el coeficiente de transferencia de materia (k) y de 40 y 52% en la D_e, tras aplicar 45 y 90 W de potencia eléctrica al transductor ultrasónico, respectivamente a 40 ºC, 1 m/s (García-Pérez y col., 2012a). Además, en el mismo trabajo, el análisis por microscopía evidenció los efectos causados por los US en la interfase sólido-gas, así como la disrupción de las células del albedo.

Los trabajos anteriormente reseñados ponen de manifiesto la influencia de la estructura del material en la eficiencia de la aplicación de US, siendo una de las variables estructurales más importantes la porosidad. Los grandes espacios intercelulares existentes en los productos altamente porosos, como la piel de naranja o limón, los hacen más sensibles a los ciclos de compresión y descompresión causados por los US, facilitando el transporte de agua a través del sólido. Al contrario, los pequeños espacios intercelulares, característicos de los productos menos porosos, como las zanahorias, dificultan la transferencia interna de materia, requiriéndose mayores niveles de energía acústica para lograr el mismo efecto (García-Pérez y col., 2009).

Una variante del equipo de secado asistido por US sin contacto permite realizar secados a baja temperatura. El trabajo, recientemente publicado (García-Pérez y col., 2012b), fue realizado en cubos de zanahoria, berenjena y manzana (10 mm), bajo presión atmosférica, a 2 m/s de velocidad de aire, -14 ºC, 7% de humedad relativa y aplicando una potencia acústica de 19,5

kW/m^3. El efecto de los US sobre la cinética de secado fue similar en todas las matrices vegetales y se obtuvo una reducción del 65 al 70% en el tiempo de proceso. El k se incrementó en un 96-170% y la D_e en un 407-428%, al aplicar potencia US, comparado con tratamientos control sin aplicar US.

Justificación y objetivos

2. JUSTIFICACIÓN Y OBJETIVOS

Durante la última década se ha producido un importante cambio en los hábitos alimentarios. Es bien conocido el interés del consumidor actual hacia alimentos que, presentando buenas características organolépticas, sean no sólo nutritivos sino también vehículos de determinados componentes y/o ingredientes con una cierta bioactividad. En este contexto, los consumidores han sido alentados a incrementar la ingesta diaria de frutas y vegetales, los cuales, según los estudios epidemiológicos existentes, han demostrado ejercer efectos beneficiosos frente a ciertas patologías tales como obesidad, enfermedades cardiovasculares, neurológicas y cáncer. Sin embargo, debido a que tanto frutas como vegetales poseen un contenido en agua superior al 80%, su período de vida útil es corto y, en la mayoría de los casos, su producción es estacional, lo que hace que sean materias primas muy adecuadas para someterlas a procesos de conservación.

Una de las opciones factibles es la deshidratación, un proceso que, si bien se conoce desde hace mucho tiempo, se encuentra en expansión por el estilo de vida actual. Los vegetales y frutas deshidratadas pueden ser fácilmente producidos y almacenados y transportados a relativamente bajo coste. Además, los productos resultantes poseen unas características diferentes a los de partida, ampliando y diversificando el mercado existente. Hasta la fecha, la mayor parte de los vegetales y frutas deshidratados se obtienen mediante secado convectivo, precedido normalmente de un tratamiento de escaldado o deshidratación osmótica. Aunque mediante este método se obtienen productos con una elevada vida útil, su calidad, en la mayoría de los casos, dista de lo que debería ofrecerse al consumidor actual, debido a los cambios químicos, físicos y físico-químicos que se originan. Además, en algunos casos se ofrecen productos con el reclamo de saludables tan sólo por el hecho de ser alimentos de origen vegetal, no existiendo estudios prospectivos en los que se evalúe la calidad nutritiva y la bioactividad de los mismos.

En el pasado, las investigaciones se centraron en la obtención de productos con una elevada vida útil sin prestar excesiva atención a mantener la calidad nutricional y organoléptica. Más recientemente, con el objeto de mejorar la eficacia del proceso y la calidad del producto final, se han llevado

a cabo numerosos estudios sobre las cinéticas de pérdida de humedad en los procesos de secado, así como sobre la optimización de los mismos. Aunque depende de numerosos factores tales como el tipo, la madurez y la geometría del producto, así como de los tratamientos previos al secado, en general, se ha visto que se requieren procesos a temperaturas relativamente altas durante varias horas. En este sentido, se han realizado trabajos sobre la relación entre los parámetros de proceso y aspectos de calidad tales como color, textura, capacidad de rehidratación, contenido nutricional y calidad sensorial. Entre los cambios químicos más estudiados destacan los que afectan a polifenoles y vitaminas, por el elevado contenido de estos constituyentes en frutas y vegetales, su susceptibilidad a las condiciones de tratamiento y su importancia nutricional. Otros cambios, tales como la interacción proteína-carbohidrato vía reacción de Maillard, especialmente en sus etapas iniciales, han sido escasamente investigados. Sin embargo, dadas las condiciones de a_w y temperatura que se alcanzan durante este proceso, dicha reacción puede originar importantes pérdidas de valor nutritivo por la participación de aminoácidos esenciales (lisina y arginina).

Para mejorar la calidad final de frutas y vegetales deshidratados, una de las alternativas que ha suscitado gran interés en los últimos años es la aplicación de tecnologías emergentes. Dentro de éstas merece una mención especial la aplicación de US de potencia. Numerosos son los trabajos que se han publicado sobre esta tecnología, que puede aplicarse tanto en sistemas líquidos, durante el tratamiento previo al proceso de secado, como en el propio proceso de secado. En el primero de los casos la mayor parte de las investigaciones se han centrado en la aplicación de US durante la deshidratación osmótica, estudiando la transferencia de materia entre el sustrato y el medio líquido empleado y las cinéticas de pérdida de humedad en el posterior proceso de secado. Hasta nuestro conocimiento, no existían trabajos previos en los que se evaluara la efectividad de los US como tratamiento de escaldado, atendiendo conjuntamente a las pérdidas por lixiviado y al efecto sobre enzimas importantes que pudieran causar deterioro durante el período de conservación.

Por lo que se refiere a la utilidad de los US durante el secado de vegetales y frutas, existen trabajos sobre la cinética de pérdida de humedad durante el proceso, que evidencian la idoneidad de dicha tecnología

emergente. Los US producen una serie de efectos como son microagitación, creación de canales microscópicos y cavitación que facilitan la eliminación del agua del interior del alimento. El efecto sinérgico de los US y la temperatura en el secado convectivo asistido por US permite llevar a cabo las deshidrataciones a menor temperatura y menor tiempo, aspectos de suma importancia para los constituyentes bioactivos y termolábiles de vegetales y frutas. Sin embargo, no se ha investigado hasta el momento, ni la calidad final del producto deshidratado ni su período de vida útil.

Así, en esta tesis doctoral cuyo punto de partida fue el proyecto SEINCADES (AGL-2007-63462), se planteó el **estudio del impacto de los ultrasonidos de potencia tanto en el escaldado como durante el proceso de secado de vegetales y frutas, prestando especial atención a las modificaciones químicas y físicas que se originan; todo ello encaminado a la obtención de vegetales y frutas deshidratados de elevada calidad que satisfagan las necesidades nutricionales del consumidor, sus preferencias y mantengan, en la medida de lo posible, la bioactividad del producto de partida.**

Para alcanzar este objetivo general se plantearon los siguientes objetivos parciales:

-Llevar a cabo un estudio en profundidad sobre la calidad de los productos deshidratados disponibles en el mercado y procedentes de industrias del sector.

-Establecer las condiciones óptimas de procesado en un prototipo de secado convectivo.

-Evaluar la viabilidad de la aplicación de US de potencia en el escaldado de vegetales.

-Estudiar los efectos de los US de potencia en el secado convectivo de vegetales y frutas, en su cinética de deshidratación y, en especial, en los principales parámetros de calidad.

Plan de trabajo

3. PLAN DE TRABAJO

Con los antecedentes previamente expuestos y con el fin de alcanzar los objetivos indicados, se abordó el siguiente PLAN DE TRABAJO, esquematizado en la **Figura 3.1**:

1. Estudio de la calidad de productos industriales (zanahoria, patata, cebolla y ajo) y comerciales (frutas comunes y tropicales) deshidratados supuestamente mediante:

    a. Secado por convección
    b. Liofilización
    c. Deshidratación osmótica

2. Secado por convección en un prototipo:

    a. Optimización de las condiciones del proceso (temperatura y velocidad de aire) y evaluación de la calidad de zanahorias deshidratadas.
    b. Estudio de la calidad de fresas deshidratadas. Cinética de la degradación de la vitamina C y de la formación de 2-furoil metil aminoácidos.

3. Estudio de la incidencia del escaldado de zanahoria mediante tratamientos convencionales y con US de potencia (baño y sonda):

    a. Efecto de la temperatura, el tiempo y la potencia sobre la inactivación enzimática y las pérdidas por lixiviado.
    b. Efecto del escaldado en la calidad y propiedades sensoriales del producto deshidratado.

4. Secado asistido por US de potencia

    a. Estudio de la calidad de zanahorias deshidratadas en un prototipo de deshidratación mediante US *por contacto*.

b. Evaluación del impacto de las condiciones de tratamiento (temperatura, potencia) en la deshidratación de fresa mediante un prototipo de secado asistido por US *sin contacto.*

b.1.Modelización matemática de la cinética de secado: aplicación de modelos difusivos y cinéticos.

b.2. Estudio de la calidad y vida útil del producto terminado.

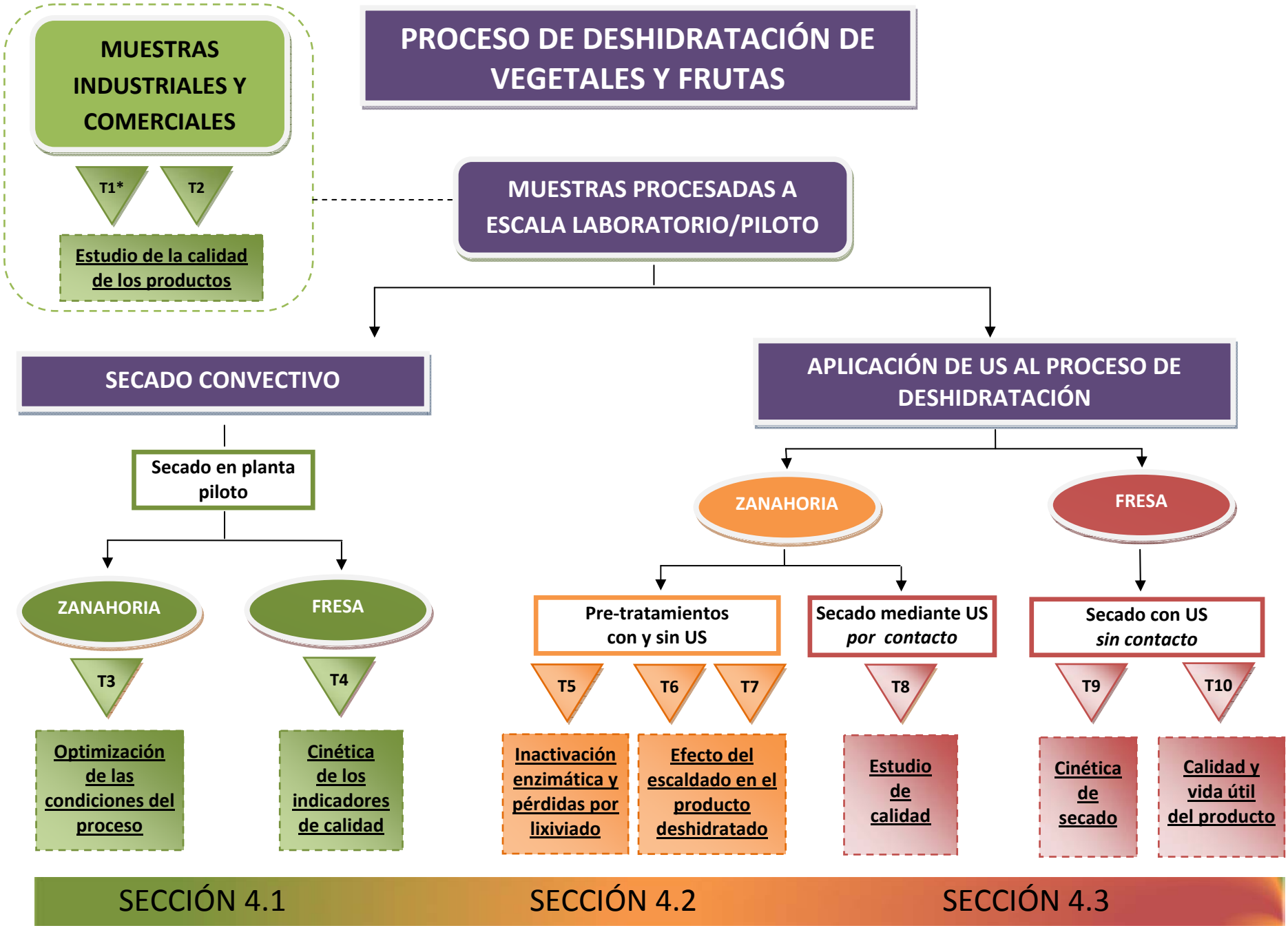

Figura 3.1 Esquema del Plan de trabajo seguido en la presente Memoria. *T1-T10: hace referencia a los trabajos mostrados en la sección de Resultados y discusión.

Resultados y discusión

4.1. Procesos convencionales de deshidratación de vegetales y frutas

4.1.1. Prefacio

Tal y como se ha indicado con anterioridad, el tratamiento de deshidratación mediante convección es el de elección en las industrias del sector que se dedican a la elaboración de vegetales y frutas deshidratadas. Aunque son numerosos los estudios que se están llevando a cabo sobre procesos alternativos al secado convencional, por el momento, ninguna de las tecnologías ensayadas ha podido desplazarlo. Los productos deshidratados que puede adquirir el consumidor en el comercio son, presumiblemente, sometidos a un proceso de secado por convección con o sin pre-tratamiento, según los casos.

Por ello, en la primera etapa del trabajo experimental llevado a cabo en la presente Memoria, se planteó un estudio de muestras industriales de vegetales proporcionadas por la empresa española Vegenat (Badajoz) (Apartado 4.1.1.1.1., *Quality parameters in industrially dehydrated vegetables during their storage*) y de frutas comerciales, adquiridos en diversos comercios españoles y europeos (Apartado 4.1.1.1.2., *Survey of quality indicators in comercial dehydrated fruits*). Para ello, se eligieron diferentes indicadores químicos y físicos que pudieran dar idea de la calidad global de los productos analizados, prestando especial atención a los indicadores de las etapas iniciales de la RM (2-FM-AA) y a las pérdidas de vitamina C. Dicha elección se basó en el hecho de que la detección precoz de los 2-FM-AA puede evitar mayores pérdidas de valor nutritivo, por la participación de la lisina y la arginina, y modificaciones en la funcionalidad de las proteínas, lo cual puede también tener incidencia en cambios físicos que afectan a la calidad sensorial del producto final. En el caso de la vitamina C, se trata de un indicador muy sensible a las condiciones de todo el proceso de deshidratación y, valores elevados de retención de la misma, pueden indicar que otros constituyentes apenas han sido afectados por el tratamiento.

En el primer estudio (Apartado 4.1.1.1.1., *Quality parameters in industrially dehydrated vegetables during their storage*) se eligieron zanahoria, patata, ajo y cebolla, por ser vegetales de gran consumo y con alto contenido en constituyentes bioactivos. Las muestras se analizaron

recién procesadas y tras 12 meses de almacenamiento bajo las condiciones habituales en las que se mantiene este tipo de alimentos durante su conservación en el mercado o en el domicilio del consumidor. Durante el proceso de deshidratación, el cambio químico más importante que se produjo fue la RM y, durante el almacenamiento, su avance fue especialmente patente en el caso de las muestras de zanahoria. Los niveles de estos compuestos fueron similares a los encontrados en la literatura para muestras comerciales de estos mismos vegetales. En general, teniendo en cuenta los indicadores químicos y físicos empleados, las muestras resultaron ser estables a lo largo del período de conservación. Especialmente interesante fue el caso de las muestras de cebolla y ajo, cuyo contenido en fructooligosacáridos (carbohidratos prebióticos) permaneció inalterado durante la conservación. La demostrada estabilidad de las muestras industriales analizadas indicó que los vegetales habían sido adecuadamente procesados en la industria.

Con similar objetivo se llevó a cabo un estudio sobre la calidad de frutas deshidratadas del comercio (Apartado 4.1.1.1.2, *Survey of quality indicators in commercial dehydrated fruits*), para lo cual se analizó un total de 30 muestras de frutas comunes y tropicales y, con fines comparativos, 2 de fresa obtenidas en el laboratorio por liofilización y convección. Lo más destacable de este estudio fue el escaso valor nutritivo (altas concentraciones de 2-FM-AA y bajas de vitamina C) y la escasa bioactividad (vitamina C) que presentaban la mayor parte de las muestras analizadas, cuya calidad distaba en gran medida de la hallada en las muestras elaboradas en el laboratorio. Con objeto de buscar un posible agrupamiento de las muestras se realizó un Análisis Cluster, teniendo en cuenta el conjunto de parámetros de calidad analizados. Los resultados de dicho análisis indicaron que las muestras se agrupaban en dos y que, en uno de los grupos, estaban incluidas las muestras que habían sido sometidas supuestamente a deshidratación osmótica previa al secado. De todas las muestras analizadas, éstas eran las que presentaban la calidad más deficiente, subrayando la importancia de los pre-tratamientos y de la selección y combinación adecuada de los indicadores para evaluar correctamente la calidad final del producto deshidratado.

Una vez conocidos algunos productos ofrecidos en el mercado, se llevaron a cabo tratamientos de deshidratación en un prototipo de secado por

convección con objeto de, empleando los indicadores de calidad usados en los trabajos anteriores, optimizar el proceso y obtener vegetales y frutas de calidad. Los trabajos subsiguientes se centraron en zanahoria y fresa, por su gran aceptación y elevado contenido en constituyentes bioactivos. En primer lugar, se procedió a la optimización de las condiciones de deshidratación de zanahoria en un prototipo de secado por convección (Apartado 4.1.1.2.1., *Optimization of convective drying of carrots using selected processing and quality indicators*). En este trabajo se realizó un diseño experimental centrado en las caras (CCD) siendo la temperatura (40-65 °C) y la velocidad del aire (2-6 m/s) las variables independientes. Las condiciones de secado empleadas, condujeron a humedades finales cercanas al 15% que aseguran la calidad microbiológica del producto final durante su período de vida útil. Se estudió la cinética de la pérdida de humedad y se identificó un primer período con velocidad constante cuando en dicha cinética se consideraba el encogimiento que sufre el producto durante el proceso. En cuanto a los parámetros de calidad (2-FM-AA y vitaminas), se observaron cambios moderados incluso en las condiciones más severas. Teniendo en cuenta como variables dependientes la pérdida de humedad durante la primera hora, la pendiente del período de velocidad constante y los parámetros de calidad anteriores, se estableció una correlación mediante un análisis por RSM (Response Surface Methodology), encontrándose que las condiciones óptimas de procesado para maximizar la función objetivo (menor dañado de constituyentes y mayor eficiencia del proceso) eran 46 °C y 4,9 m/s.

Una vez conocidas las condiciones experimentales del equipo de secado por convección, otro de los objetivos dentro de esta primera fase fue deshidratar fresa en dicho prototipo (Apartado 4.1.1.2.2., *Impact of processing conditions on the kinetics of vitamin C degradation and 2-furoylmethyl amino acid formation in dried strawberries*). Se efectuaron tratamientos a temperaturas de 40 a 70 °C, velocidades de aire 2-8 m/s y tiempos de secado de 1 a 7 h, y se estudió la cinética de pérdida de la vitamina C, especialmente abundante en esta fruta, y la de formación de 2-FM-AA. Como es sabido, una forma de evitar el deterioro de determinados constituyentes de los alimentos es a través del conocimiento de la cinética de las reacciones implicadas. La dependencia de ambas reacciones con respecto a la temperatura se demostró mediante la ecuación de Arrhenius, encontrándose

valores de E_a de 82,1 kJ/moL para vitamina C y 55,9 y 58,2 kJ/moL para 2-FM-GABA y 2-FM-Lys + 2-FM-Arg, respectivamente. Además, ambos tipos de indicadores se correlacionaron mediante una regresión lineal simple con valores de R superiores a 0,96. Cabe destacar que los 2-FM-AA encontrados fueron detectados por primera vez en fresa sometida a procesos de deshidratación.

4.1.1.1 Estudio de muestras industriales y comerciales

4.1.1.1.1 Parámetros de calidad en vegetales deshidratados industrialmente durante su almacenamiento

Quality parameters in industrially dehydrated vegetables during their storage

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Journal of Food and Nutrition Research, 51 (3), 2012, 132-144

Abstract

A comprehensive study on physical and chemical quality parameters (dry matter, water activity, major and minor carbohydrates, 2-furoylmethyl amino acids (2-FM-AA), as indirect indicators of initial steps of Maillard reaction (MR), proteins, total polyphenol content and rehydration ratio) has been carried out on several highly consumed vegetables industrially dehydrated (carrot, onion, garlic and potato). After processing**,** the main observed change was the formation of 2-FM-AA, mainly in carrot samples, indicating the participation of amino acids as lysine during the MR evolution. With respect to the effect of 12-month storage under conditions usually used by consumers (in the dark, 19-27 ºC, 15-41% relative humidity), with the exception of carrots, no remarkable amounts of 2-FM-AA was generated, in agreement with the slight variation in proteins pattern and carbohydrate composition. Particularly interesting is the case of onion and garlic which preserve during storage their content in prebiotic carbohydrates. Samples were also stable with regard to their polyphenol content and rehydration ability, showing the importance of sample pre-treatment, processing and storage conditions for preservation of bioactivity and overall quality of dehydrated vegetables. These results underline the usefulness of the indicators here used and these data could be valuable for technologists, nutritionists and consumers.

Introduction

Nowadays, consumers are highly interested in processed foods which fulfil not only their nutritional requirements, but also which provide them with health benefits. Preservation of quality and easy handling and storage, particularly under non-refrigerated conditions, are also a consumer demand. Thus, and with a view to obtain processed foods of premium quality with preserved functionality, food processing industries are making a considerable effort in the improvement of existing technologies through optimization of process design.

Drying, which decreases the water content of the raw product to the level that minimizes its biochemical, chemical and microbiological deterioration, is one of the oldest methods of food preservation and represents a very important process in the food industry (Doymaz, 2008b). Forced convection by hot air is the most common industrial technique to perform food drying, being drying temperature and time, air velocity and relative humidity, as well as the initial moisture content of the product, the most relevant process factors (Gowen et al., 2008; Lewicki, 2006). Convective drying can be carried out at high temperatures for short times or at lower temperatures for longer times; the former option being usually preferred since it produces less thermal damage and consumes less energy (Velic et al., 2004). Simplicity of operation and affordable technology are other additional advantages of convective drying for industrial food processing.

Among the different foods that can become dehydrated, vegetables hold a predominant position as they can be consumed either on their own or as ingredients for the elaboration of other food products such as soups, sauces, etc. Thus, the demand of dehydrated vegetables has considerably increased over the last few years in many countries and it is expected to increase even further during the next decade (Zhang et al., 2006).

Dehydration by hot air may cause a series of chemical, physicochemical, physical and biological alterations that can affect the final quality of the dehydrated vegetable. One of such chemical modifications which can take place if dehydrated vegetables are submitted to intensive treatment and/or inappropriate storage is Maillard reaction (MR). MR takes place between the

carbonyl group from reducing carbohydrates and the free amino group of amino acids, peptides or proteins. In advanced stages of MR, a loss in the nutritional value of the food may occur and the development of undesirable coloured and fluorescent compounds, together with the formation of new volatile compounds, can alter the organoleptic properties of the product (Villamiel et al., 2006). Therefore, the evaluation of the initial stages of this reaction provides very valuable information for optimization of food processing, since it allows controlling this reaction before important nutritional and/or organoleptic changes take place in the dehydrated food. In this respect, quality indicators derived from the initial stages of MR (2-furoylmethyl amino acids, 2-FM-AA) have been previously investigated in dehydrated samples of garlic, onion and carrot (Cardelle-Cobas et al., 2005; Rufián-Henares et al., 2008; Soria et al., 2009b). These quality markers had previously being proved highly valuable in the study of other processed foods of vegetable origin such as dehydrated fruits, jams and fruit-based infant foods and processed tomato products (Sanz et al., 2000; 2001; Rada-Mendoza et al., 2004; Cardelle-Cobas et al., 2009).

In addition, dehydration produces shrinkage and may affect negatively the rehydration ability of dehydrated vegetables (Lewicki, 2006; Cardelle-Cobas et al., 2009). This is due to a series of factors related to physical and physicochemical changes occurring in the tissues (Sabater-Molina et al., 2009), and also to chemical changes that might affect carbohydrates and proteins (Panyawong & Devahastin, 2007).

At the sight of the above exposed, in this paper, a comprehensive study on quality parameters including major and minor carbohydrates, 2-FM-AA, proteins, polyphenols and rehydration capacity has been carried out in highly consumed industrially dehydrated vegetables such as potato, carrot, onion and garlic. The changes in these parameters with storage under conditions normally used by consumers have also been assessed.

Materials and methods

Samples

Six industrially dehydrated samples of carrot (*Daucus carota*), onion (*Allium cepa)*, garlic (*Allium sativum)* and potato (*Solanum tuberosum*) kindly provided by a Spanish vegetable products company (Vegenat, Badajoz, Spain) were studied. Two geometries of carrot products: cubes and flakes (carrots I and II, respectively), and two sizes of onion flakes (small, onion I and large, onion II), were analysed together with garlic and potato flakes. Industrial processing conditions, summarized under **Table 4.1**, mainly consisted of a blanching step with hot water spray (microdroplets) prior to either one or two dehydration stages.

Table 4.1 Industrial processing conditions of dehydrated vegetables under study.

Sample code	Blanching		Dehydration			
			1st Stage		2nd Stage	
	t [min]	T [ºC]	t [h]	T [ºC]	t [h]	T [ºC]
Carrot I	20.0	98	3.0	65-135	2.0	58
Carrot II	20.0	98	5.0	55-135	-	-
Onion I	3.0	98	6.5	50-125	-	-
Onion II	3.0	98	6.5	50-125	-	-
Garlic	-	-	5.5	58-120	-	-
Potato	30.0	98	4.5	50-125	-	-

Storage assays

Dehydrated samples, packed in polypropylene individual bags (30 mm thick sample layer) and sealed, were stored in the dark for a period of 12 months under the following ambient conditions: temperature between 19.3 ºC and 27.1 ºC; relative humidity between 15.0% and 40.7%. After 6 and 12 months of storage, samples were taken and stored frozen at -20 ºC until analysis.

Characterization of samples

The dry matter (DM) content was determined gravimetrically by drying the samples until constant weight according to the AOAC method (1990a).

Water activity (a_w) measurement was carried out in a Novasin a_w Sprint TH-500 equipment (Pfäffikon, Switzerland). Saturated aqueous solutions of LiCl, $MgCl_2$, $Mg(NO_3)_2$, NaCl, $BaCl_2$ and $K_2Cr_2O_7$ were used to calibrate the sensor unit. The Kjeldahl method was performed to determine total nitrogen (TN) using 6.25 as conversion factor (TN x 6.25) (AOAC, 1990b). All determinations were carried out in duplicate.

Rehydration ratio (RR)

Rehydration of industrially processed samples was performed in distilled water (solid-to-liquid ratio 1:50) according to Soria et al. (2010). Dried samples were rehydrated by immersion in water at 20 ºC for 24 hours. Vegetables were placed onto paper towels to remove the surface water and further weighed. Each rehydration experiment was performed in duplicate and RR was calculated as:

$$RR = m_r/m_d \quad (1)$$

where m_r and m_d are the weights of the rehydrated and the dehydrated vegetable, respectively.

Analytical determinations

HPLC analysis of 2-furoylmethyl amino acids

Analysis of 2-FM-AA was carried out by ion-pair RP-HPLC (Resmini & Pellegrino, 1991). A C_8 column (250 mm x 4.6 mm i.d.) (Alltech, Lexington, Kentucky, USA) thermostated at 37 ºC was used, with a linear binary gradient (A, 4 mL/L acetic acid; B, 3 g/L KCl in A) and a variable-wavelength detector at 280 nm (LCD Analytical SM 4000, LCD, Riviera Beach, Florida, USA). The elution programme was as follows: 100% A from 0 to 12 min, 50% A from 20 to 22.5 min, and 100% A from 24.5 to 30 min.

Samples (0.25 g) were hydrolysed under inert conditions (helium) with 4 mL of 8 M HCl at 110 ºC for 23 h in a screw-capped Pyrex vial provided with a PTFE-faced septum. A medium-grade paper filter (Whatman no. 40, General Electric Company, Fairfield, Connecticut, USA) was used to filter the sample hydrolysate and then, 0.5 mL of the filtrate was applied to a Sep-Pack

C_{18} cartridge (Millipore, Billerica, Massachusetts, USA) previously activated with 5 mL of methanol and 10 mL of distilled water. 3 mL of 3 M HCl were used to elute the retained compounds from the Sep-Pack cartridge and only 50 µL were injected into the HPLC system.

Data obtained for standards previously synthesized in our laboratory and analysed under identical experimental conditions were used to identify 2-FM-AA other than furosine (2-FM-lysine) (Sanz et al., 2001). Quantitation was performed by the external standard method, using a commercial standard of 2-FM-lysine (Neosystem Laboratoire, Strasbourg, France). Data shown in this paper (expressed as milligrams per kilogram of protein) are the mean values of two replicates.

GC analysis of carbohydrates

Soluble carbohydrates were extracted in duplicate according to the method described by Soria et al. (2010). Dehydrated vegetables were frozen prior to grinding to powders using a laboratory mill IKA A-10 (IKA Labortechnik, Staufen, Germany). Samples (30 mg) were weighted into a polyethylene tube and extracted at room temperature with 2 mL of Milli-Q water (Millipore) under constant stirring (50 Hz) for 20 min. Then, 8 mL of absolute ethanol were added followed by 0.2 mL of an ethanolic solution 10 mg/mL of phenyl-β-D-glucoside (Sigma-Aldrich Chemical, St. Louis, Missouri, USA) used as internal standard. After stirring for 10 min, samples were centrifuged at 10 °C and 9600*g* for 10 min and the supernatant was collected. Precipitates were submitted to a second extraction with 10 mL of 80% ethanol under the same conditions to obtain recovery values close to 100%. Finally, an aliquot (2 mL) of supernatant was evaporated under vacuum at 40 °C.

GC analyses were performed with an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with a flame ionization detector (FID), using nitrogen as carrier gas at a flow rate of 1 mL/min. The trimethylsilyl oxime (TMSO) derivatives, prepared as described by Soria et al. (2010), were separated using two different methods according to the sample composition. For carrot and potato samples, the analysis was performed as described by Soria et al. (2010): the TMSO were

separated using an HP-5MS fused silica capillary column (30 m long x 0.25 mm i.d. x 0.25 µm film thickness) coated with 5% phenylmethylsilicone (J&W Scientific, Folsom, California, USA). The oven temperature was held at 200 ºC for 11 min, raised to 270 ºC at a heating rate of 15 ºC/min, raised again to 300 ºC at 3 ºC/min, and finally raised to 315 ºC at 15 ºC/min, remaining at this temperature for 3 min. The injector (split ratio 1:40) and detector temperatures were 280 ºC and 315 ºC, respectively.

For onion and garlic samples, analyses were carried out as described by Montilla et al. (2006), using a WCOT fused silica capillary column (Chrompack, Middelburg, The Netherlands). The column (8 m long x 0.25 mm i.d. x 0.25 µm film thickness) was coated with 5% diphenyl 95% dimethylsilicone (HT-5, Supelco, Sigma-Aldrich). Injector (split ratio 1:10) and detector temperatures were 280 °C and 360 °C, respectively. The initial oven temperature was 100 °C, raised to 250 ºC at a heating rate of 10 °C/min, and raised again to 360 °C at 5 °C/min and holding at this temperature for 5 min.

Data acquisition and integration were performed using Agilent ChemStation Rev. B.03.01 software (Wilmington, Delaware, USA). The identification of TMSO derivatives of carbohydrates was carried out by comparing the experimental retention indices with those of standards previously derivatized. Quantitative data (in grams per kilogram of dry matter, DM) were calculated from FID peak areas. Standard solutions of glucose, fructose, saccharose, *myo*-inositol, *scyllo*-inositol, kestose and nystose (all of them from Sigma-Aldrich) over the expected concentration range in vegetable extracts (0.01-5 mg/mL) were prepared to calculate the response factor relative to phenyl-β-D-glucoside. Response factor of kestose and nystose were applied for quantitation of trisaccharides and oligosaccharides with degree of polymerisation (DP) ≥ 4, respectively.

Total polyphenol content (TPC)

To obtain methanolic extracts, 2.5 mL of HPLC grade methanol were added to 0.1 g of sample powders and the mixture was then homogeneized for 1 min at 60 Hz with an Ultra-Turrax T-25 homogenizer (IKA Labortechnik). After stirring with a Thermomixer (Eppendorf, Hamburg,

Germany) for 20 min at 50 Hz, samples were centrifugated for 15 min at 2000*g*. The supernatants were then filtered through 0.45 µm PVDF Acrodisc syringe filters (Sigma-Aldrich) for subsequent Folin-Ciocalteau determination.

TPC was determined according to Singelton et al. (1999) and to Patras et al. (2009), with slight modifications. 100 µL of filtered methanolic extract, 100 µL of methanol and 100 µL of Folin-Ciocalteau reagent (2 M, Sigma-Aldrich) were mixed in a 2.0 mL eppendorf tubes. Five minutes later, 700 µL of 75 g/L Na_2CO_3 were added and the samples were vortexed briefly. After 20 min in the dark at room temperature, the samples were centrifugated at 10000*g* for 3 min. The absorbance of the samples was read at 735 nm, using gallic acid solutions (10-400 mg, Sigma-Aldrich) as standards. Results were expressed as grams of gallic acid equivalent (GAE) per kilogram of dry matter.

SDS-PAGE analysis of protein isolates

100 mg of dehydrated sample powders were mixed with 2 mL of 1% sodium metabisulfite (Merck, Darmstadt, Germany) aqueous solution. Next, samples were stirred thoroughly for 2 h and centrifugated at 3,000*g* for 15 min. The supernants were finally analysed by SDS-PAGE.

Protein analysis was carried out by adding 32.5 µL of sample supernant to12.5 µL of 4X NuPAGE LSD sample buffer (Invitrogen, Carlsbad, California, USA) provided with 5 µL of 0.5 M dithiothreitol (DTT, Sigma-Aldrich). Samples were heated at 70 ºC for 10 min and 20 µL were loaded on a 12% polyacrylamide NuPAGE Nove Bis-Tris precast gel (Invitrogen). Gels were run for 41 min at 120 mA per gel and 200 V with a continuous MES SDS running buffer (Invitrogen) and were stained using the Colloidal Blue Staining Kit (Invitrogen). A mixture of standard proteins with relative molecular weight ranging from 2.5 to 200 kDa (Invitrogen) was used to estimate the molecular weight of proteins. Myosin, 200 kDa; β-galactosidase, 116.3 kDa; phosphorylase B, 97.4 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55.4 kDa; lactate dehydrogenase, 36.5 kDa; carbonic anydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa; aprotinin, 6 kDa; insulin B chain, 3.5 kDa and insulin A chain, 2.5 kDa were chosen as standards.

Results and discussion

Protein, moisture, a_w and rehydration ratio of dehydrated samples

Table 4.2 shows the initial percentage of protein and evolution of moisture, a_w and rehydration ratio during the 12-month storage of industrially dehydrated carrot, onion, garlic and potato samples under study.

Table 4.2 Protein content and moisture, a_w and rehydration ratio before and after 12 months of storage

Sample code	Protein [%]	Moisture [%]		a_w		Rehydration ratio [%]	
		0 m	12 m	0 m	12 m	0 m	12 m
Carrot I	11.14	9.25	10.41	0.320	0.337	5.1	5.1
Carrot II	6.40	7.22	9.24	0.303	0.333	7.5	7.6
Onion I	8.62	7.39	8.80	0.295	0.341	3.2	3.2
Onion II	8.41	6.40	8.21	0.275	0.345	3.5	3.4
Garlic	19.13	7.77	8.09	0.312	0.372	2.6	2.6
Potato	6.82	7.84	8.10	0.277	0.351	3.8	3.9

The protein values here determined were similar to data reported in the literature (Cardelle-Cobas et al., 2005; Souci et al., 1987). The highest protein content corresponding to the dehydrated garlic sample. The initial moisture of the studied samples ranged from 6.4% to 9.2%, values that are close to those previously reported for different dehydrated vegetables (USDA, 2011). Although after 12 months of storage, a slight increase in moisture was observed (8.1-10.4%), these levels were within the permissible limit to assure the microbiological stability of dehydrated vegetables (12-15%) (Belitz et al., 2009a). Rahman et al. (2010), in a study on solar dried carrots, found a higher increase (from 7.05% to 16.22%) in the moisture content of these samples after 8 months of storage. These differences could be probably attributed to the different sample geometry and humidity conditions used in both assays.

At the beginning of the storage, a_w values of all dehydrated vegetables analysed were within the range 0.275-0.320. The different samples presented slight differences in the initial values of $a_{w,}$ probably due to the different processing conditions, and during their storage they only suffered a slight increase in their a_w values (0.333-0.372), according to our results of dry matter evolution. As it is well known, dried foods with a_w values close to

0.3 are stable against non-enzymatic browning, enzymatic activities and the development of microorganisms (Labuza, 1971; Lavelli et al., 2007). In green onion dried at 50-70 °C, García et al. (2010) found a_w values in the range 0.29-0.40 which increased up to 0.62-0.65 after a period of storage of 126 days at room temperature. The higher relative humidity in those assays (50-75%) must be considered for interpretation of these a_w data.

Regarding the rehydration ability, as observed in **Table 4.2**, data in the range 2.6-7.5 were found for all the dehydrated samples analysed, the highest value being that of carrot II and the lowest corresponding to garlic. Whereas no noticeable differences were observed in RR of onions with different sample size submitted to identical dehydration process, very unlike results were observed in rehydration of carrots I and II. These differences could be mainly attributed to the different sample geometry (cubes and flakes) and/or processing conditions employed in dehydration, among other factors. For carrot, Soria et al. (2010) reported RR values within the range 4.7-8.0 in dehydrated samples subjected to different processing conditions. In agreement with the scarce change in the moisture content during storage, hardly any variation in the RR was observed, indicating the stability during 12 months of the physical structure of the dehydrated carrot, onion, potato and garlic samples analysed. According with this, instability of solar dried carrots after 8 months of storage at ambient temperature, conditions giving rise to moisture values near 16%, could be responsible for the decrease in the RR observed for this dehydrated vegetable by Rahman et al. (2010).

Total polyphenol content

Vegetables are well known for their antioxidant activity which is, in great part, attributed to the polyphenol content. In fact, a linear correlation has been observed between polyphenolic compounds and (hydrophilic) antioxidant activity of several fruits and vegetables (Bennett et al., 2011; Netzel et al., 2007).

As an indicator of antioxidant activity, TPC (expressed as GAE) determined in industrially dehydrated carrot, onion, garlic and potato samples is shown in **Figure 4.1**. As expected, TPC was variable according to the vegetable composition; the highest phenolic content being found in carrots

and the lowest in potato sample. Regarding carrots I and II, different TPC was found for both types of sample. The different fresh carrot variety and/or maturity stage, probably associated with an unlike polyphenol content, together with the differences in sample processing (**Table 4.1**), could explain these results. As it is known, TPC in vegetables has been described to be influenced by a number of factors, including genetic variety or cultivar, soil condition, water availability, season, degree of maturity, processing, etc. (Alasalvar et al., 2001; Gorinstein et al., 2009; Soria et al., 2010; Yang et al., 2010; Bennett et al., 2011; Patras et al., 2011; Pérez-Gregorio et al., 2011).

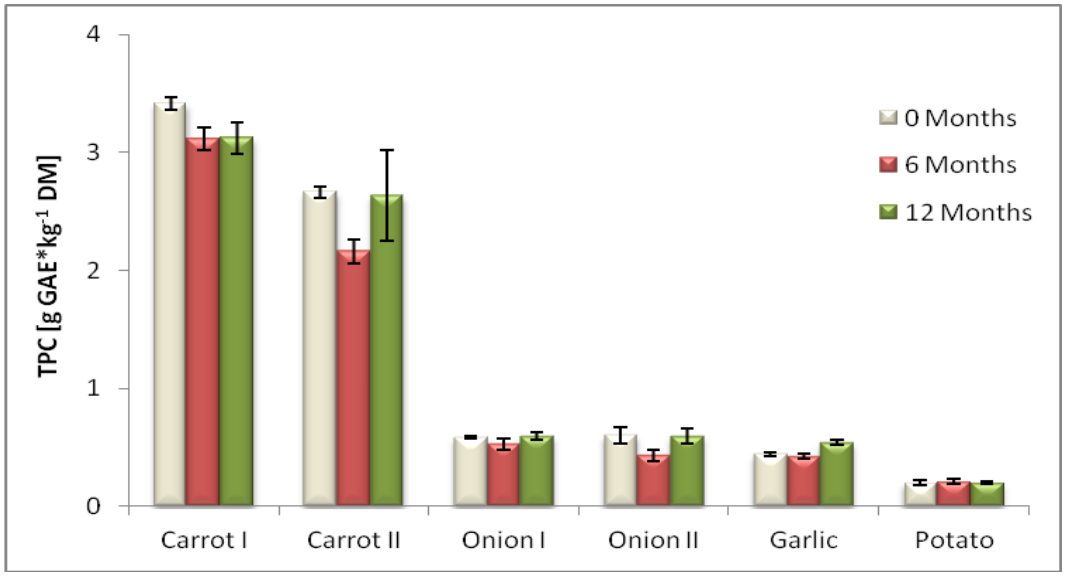

Figure 4.1 Effect of storage on total polyphenol content (TPC) of industrially dehydrated carrot, onion, garlic and potato samples. TPC is expressed as gallic acid equivalents (GAE) in grams per kilogram of dry matter.

In order to investigate the stability of TPC, its evolution was assessed during the storage. In general, after 12 months hardly any change was observed in TPC values of assayed vegetable samples. Similarly, Pérez-Gregorio et al. (2011) studied the evolution of flavonols and anthocyanins in freeze-dried onions stored at room temperature in absence of light, and no changes were observed during the 6 months of storage. In agreement with this, no change if any, in TPC and antioxidant activity was also found by Bennett et al. (2011) in dried fruits during their storage at 21 °C for 5 months. In the present paper, the blanching pre-treatment (98 °C for 3-30 min, **Table 4.1**) of fresh vegetables prior to drying seems to be efficient to

control polyphenol oxidation by polyphenol oxidases and peroxidases, the main enzymes responsible for quality loss during storage of vegetables (Kumar et al., 2001; Tomás-Barberán et al., 2001).

Maillard reaction evolution

Table 4.3 lists the results of the initial values of 2-furoylmethyl (2-FM) derivatives and their evolution during the storage of dried vegetables under study. In agreement with the amino acids present in these vegetables (USDA, 2011), 2-FM-alanine (2-FM-Ala), 2-FM-γ-amino butyric acid (2-FM-GABA), 2-FM-lysine (2-FM-Lys) and 2-FM-arginine (2-FM-Arg) were detected depending on the studied vegetable. Regardless of the processing conditions and the protein content of the sample, the highest initial 2-FM-AA values were observed in carrot, whereas the lowest were found in garlic and potato samples, probably due to the lower content in reducing carbohydrates, as compared to that of the other vegetables. In the case of carrot, similar values were reported by Soria et al. (2009b) for commercial dehydrated samples. However, for onion samples, in the present paper, lower 2-FM-derivatives were detected in comparison to data reported by Cardelle-Cobas et al. (2005), probably due to differences in the dehydration process, carbohydrate content, among other factors.

Table 4.3 Evolution with 12 month-storage of the 2-FM-AA content of dehydrated vegetables under analysis

Samples	2-FM-AA [mg·kg^{-1}]*								
	2-FM-Ala			2-FM-GABA			2-FM-Lys + 2-FM-Arg		
	0 months	6 months	12 months	0 months	6 months	12 months	0 months	6 months	12 months
Carrot I	216.2±34	346.4±136	643.5±59	278.7±16	374.4±90	586.0±22	447.5±11	435.2±39	556.4±37
Carrot II	118.0±9	542.2±122	672.1±77	226.0±16	336.0±88	708.9±24	422.8±22	578.8±82	931.6±87
Onion I	-	-	-	-	-	-	74.4±17	72.8±13	96.2±6
Onion II	-	-	-	-	-	-	112.4±11	132.5±13	146.8±7
Garlic	-	-	-	-	-	-	8.1 ± 0.6	7.9 ± 0.8	8.0 ± 0.5
Potato	-	-	-	-	-	-	83.9±9	50.4±7	66.3±9

*Content of 2-FM-AA is expressed per kilogram of protein. Values represent mean ± standard deviation, n = 2.

With the exception of dehydrated carrot samples, under the conditions used during storage period, hardly any effect on 2-FM-AA formation was observed, since MR proceeds slowly at ambient temperature and low a_w and generally requires months before substantial browning is observed. Cardelle-Cobas et al. (2005) reported a considerable increase of 2-FM-AA content when a sample of onion was stored under inappropriate conditions during two days (50 ºC, a_w 0.44). In spite of the evolution of MR in carrot samples analysed in the present study, the levels of 2-FM-Ala, 2-FM-GABA and 2-FM-Lys + 2-FM-Arg were within the range previously reported by Soria et al. (2009b) for commercial samples.

In addition to this, it is well known that MR might potentially enhance the antioxidant activity of foods (Yilmaz & Toledo, 2005). Moreno et al. (2006), in a study on the storage of dehydrated onion and garlic samples, demonstrated that whereas the Amadori compounds originated in the first steps of MR might exert a moderate effect on the antioxidant activity, the advanced Maillard products are the major contributors to this property. In this respect, the slight evolution of MR during the storage of dehydrated vegetables analysed in the present study does not contribute to changes in their antioxidant activity.

SDS-PAGE analysis of proteins

Figure 4.2 depicts the SDS-PAGE profiles of all vegetables analysed before and after 12 months of storage. Each vegetable presented a different pattern of electrophoretic bands, corresponding to the different fractions of proteins found in each specie.

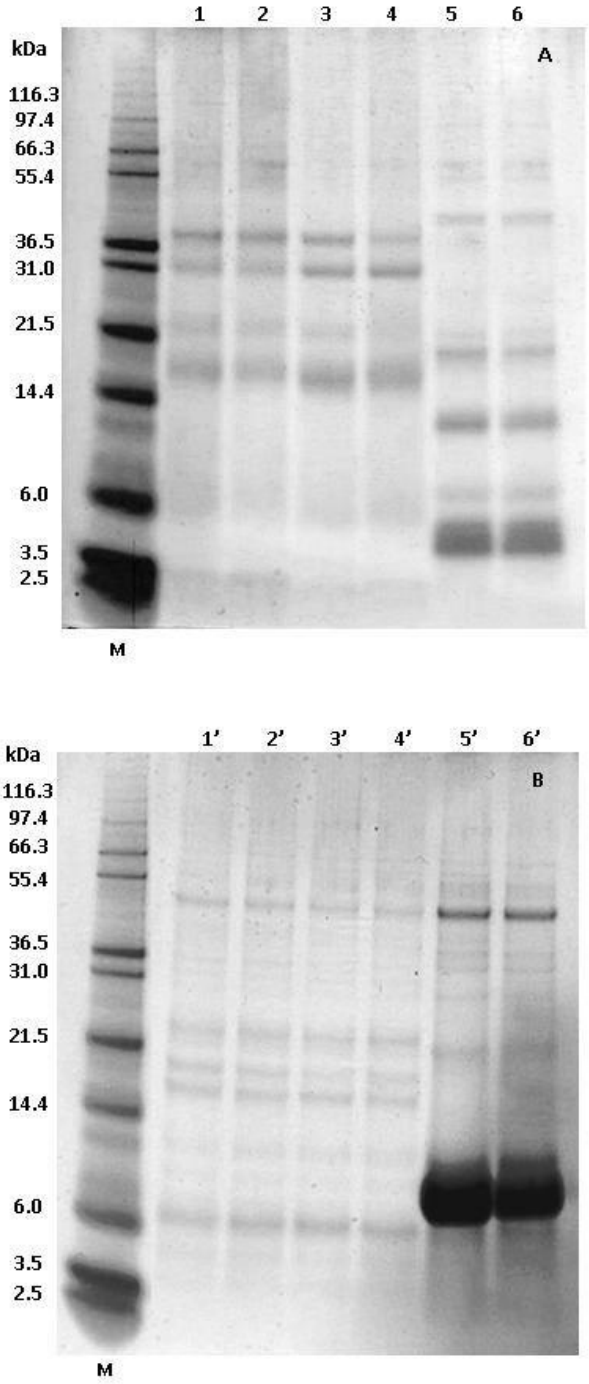

Figure 4.2 SDS-PAGE analysis of (A) dehydrated carrot and potato samples and (B) dehydrated onion and garlic samples before and after 12-month storage: (1) Carrot I, 0 months; (2) Carrot I, 12 months; (3) Carrot II, 0 months; (4) Carrot II, 12 months; (5) Potato, 0 months; (6) Potato, 12 months; (1') Onion II, 0 months; (2') Onion II, 12 months; (3') Onion I, 0 months; (4') Onion I, 12 months; (5') Garlic, 0 months; (6') Garlic, 12 months. (M) Markers of molecular weight.

All carrot samples analysed (**Figure 4.2A**, lanes 1-4) showed a profile consisting mainly of four bands with molecular weight (MW) of ~ 18 kDa, 22 kDa, 31 kDa and 41.2 kDa, very similar to that found by Soria et al. (2010) for commercial dehydrated carrot samples. However, this was slightly different to that of freeze-dried carrots, reported by the same authors; differences being probably attributed to structural modifications in protein taking place during hot air-drying. Potato samples (**Figure 4.2A**, lanes 5 and 6) showed a band with MW of ~ 42 kDa, probably corresponding to patatin, and two bands with MW of ~ 10 and 20 kDa, presumably being serine protease inhibitors PI-1 and PI-2 and potato Kunitz-type protease inhibitor, respectively. Moreover, potato profiles also showed an intense band (as a doublet) with MW < 6 kDa, which could correspond to derivatives from age-associated proteolysis due to cysteine-proteases activity. This electrophoretic profile was consistent with that recently found by Weeda et al. (2011) for fresh samples stored at 4 °C during 4-22 months. Onion samples (Figure 4.2B, lanes 1'-4') showed mainly five bands with MW of ~ 6 kDa, 17.9 kDa, 19.5 kDa, 23 kDa and 50 kDa, similar to those found by Herrera-Corredor & Carrillo-Castañeda (2007) for fresh seed onion samples. Finally, profiles of garlic samples (**Figure 4.2B**, lanes 5' and 6') were characterized by one intense band having a MW of ~ 7-13 kDa, presumably corresponding to allivin, similar to that detected by Wang & Ng (2001) in fresh bulb samples. In addition, in garlic dehydrated samples, other band with a molecular weight over 30 kDa could also be clearly seen. This might correspond to a chitinase similar to those isolated from leek (*Allium porrum*) (Vergauwen et al., 1998). Gorinstein et al. (2008) obtained electrophoretic patterns of raw red onion and garlic samples with specific bands in the 50 kDa to 112 kDa range of molecular mass that disappeared after boiling for more than 20 min, indicating that the least stable proteins (superoxide dismutase, among others) of these vegetables can be affected during processing.

With the exception of carrot samples, to the best of our knowledge, no works on the protein profiles of dehydrated samples have been previously reported. With respect to the stored samples, similar electrophoretic profiles as compared to the initial samples, were found and no protein aggregates of high molecular weight were observed. This is indicative of the scarce degree

of protein degradation during storage as a result of MR, in agreement with the low levels of 2-FM-AA listed in **Table 4.3**.

Carbohydrate analysis

As far as carbohydrate composition of dehydrated vegetables is concerned, different GC profiles were found depending on the studied vegetable specie. As an example, **Figure 4.3** shows that corresponding to onion I. A similar profile with lower total carbohydrate amount was obtained for garlic sample. In both onion and garlic, together with mono- and disaccharides, other carbohydrates with higher molecular weight were also detected. However, only a very small peak in the elution region of trisaccharides was found in carrot samples and most of carbohydrates present in these samples were mono- and disaccharides. Dehydrated potato was the sample with the simplest carbohydrate chromatographic profile.

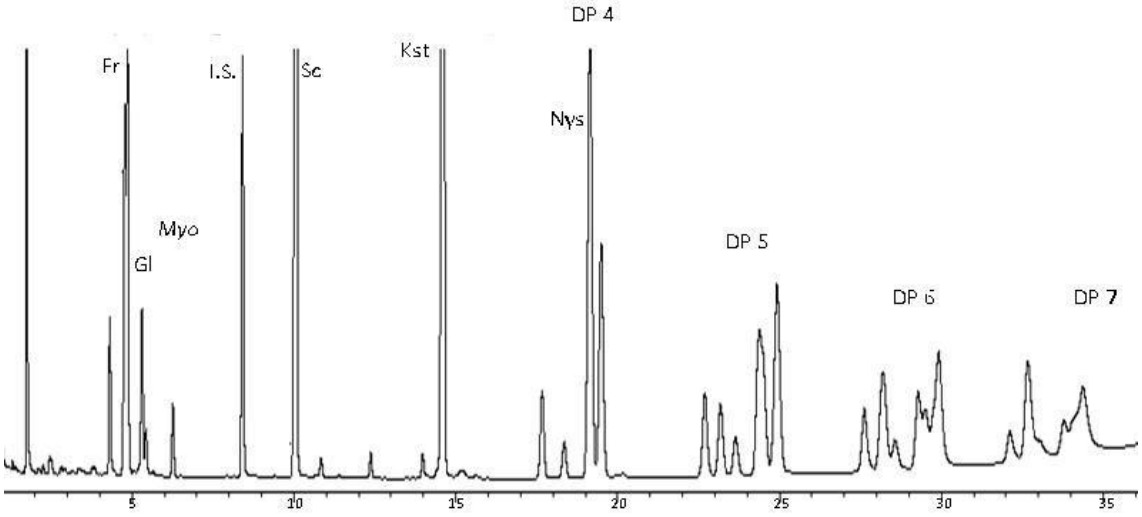

Figure 4.3 Gas chromatographic profile of the TMSO derivatives of carbohydrates present in onion I. Peaks are labelled as follows: Fr, fructose; Gl, glucose; Myo, *myo*-inositol; I.S, phenyl-β-D-glucoside (internal standard); Sc, saccharose; Kst, kestose; Nys, nystose; DP 4, tetrafructooligosaccharides; DP 5, pentafructooligosaccharides, DP 6, hexafructooligosaccharides; DP 7, heptafructooligosaccharides (DP, degree of polymerisation).

Quantitative results of carbohydrate analysis of dehydrated carrot, potato, onion and garlic samples after storage for 12 months are listed in **Tables 4.4** and **4.5**. In carrot samples, fructose, glucose and saccharose

were the major carbohydrates. Other minor carbohydrates such as the polyalcohols *scyllo*- and *myo*-inositol and the higher-carbon monosaccharide sedoheptulose were also present in both carrot I and II. The slight differences observed within the two carrots analysed could be mainly due to the different variety and/or maturity stage of raw samples. The obtained results are in agreement with quantitative ranges reported by Soria et al. (2009a) for commercial hot air-dried carrots. In the case of potato sample, as can be observed, fructose, glucose and saccharose were found in very low amounts (1-4 $g \cdot kg^{-1}$ DM), according to data previously reported by McDonald & Newson (1970) and Li et al. (2002).

As observed in **Table 4.5**, a great difference in the carbohydrate content was detected among onion and garlic dehydrated samples here analysed. In onion samples, mono- and disaccharides were present, together with high levels of kestose, nystose and other unidentified fructooligosaccharides (FOS) with degree of polymerisation 4-7. As compared with data previously reported by other authors (Cardelle-Cobas et al., 2005; Muir et al., 2009), the amount of fructose and glucose in both onion samples was significantly lower. Darbyshire & Henry (1979), in a study on different onion cultivars, found a large variability in the content of fructose, glucose, saccharose and FOS, ranging from 21-164 g/kg DM, 7-200 g/kg DM, 57-157 g/kg DM, and 200-800 g/kg DM, respectively. In agreement with this, Kahane et al. (2001) detected different content of fructose (2-45%), glucose (1-40%), saccharose (12-22%), and FOS (0-70%) respect to the amount of total carbohydrates, in different onion varieties. In addition to the variety, Muir et al. (2009) also reported a great variability in carbohydrate composition of vegetables, according to their degree of ripeness.

Table 4.4 Effect of storage on major and minor carbohydrates content of dehydrated carrot and potato samples

Carbohydrates [g·kg^{-1}]*								
Sample	**Fructose**	**Glucose**	**Saccharose**	***Scyllo*-inositol**	***Myo*-inositol**	**Sedoheptulose**	**Trisaccharides**	**Total carbohydrates**
Carrot I								
0 months	41.9±0.2	22.7±0.2	352.9±0.3	2.4±0.3	4.1±0.0	7.5±0.1	5.4±0.4	436.6±0.0
6 months	46.1±0.1	23.1±0.3	371.7±3.4	2.0±0.2	3.9±0.0	8.2±0.3	5.0±0.0	459.9±3.1
12 months	46.6±1.5	23.5±0.1	411.4±0.4	2.5±0.1	4.6±0.1	2.5±0.1	5.9±0.1	502.2±2.6
Carrot II								
0 months	48.1±2.0	36.0±2.5	381.3±12.1	2.6±0.0	4.9±0.2	10.1±0.7	7.9±0.3	490.8±17.1
6 months	46.8±0.3	36.2±0.3	383.7±4.6	2.1±0.2	4.5±0.1	12.3±0.2	8.8±0.2	494.5±5.8
12 months	53.2±0.3	43.4±0.3	404.6±1.9	3.8±0.1	5.7±0.0	11.7±0.1	9.3±0.2	531.7±1.8
Potato								
0 months	1.2±0.0	0.9±0.1	4.4±0.0	-	0.3±0.0	-	0.3±0.0	7.1±0.0
6 months	1.6±0.1	1.1±0.1	3.8±0.0	-	0.4±0.0	-	0.2±0.0	7.1±0.0
12 months	2.9±0.2	1.9±0.1	4.0±0.0	-	0.4±0.0	-	0.2±0.0	9.4±0.3

*Content of carbohydrates is expressed per kilogram of dry matter. Values represent mean ± standard deviation, n = 2.

Table 4.5 Effect of storage on major and minor carbohydrates content of dehydrated onion and garlic samples

Sample	Carbohydrates [g·kg^{-1}]*										
	Fru	**Glu**	***Myo***	**Sac**	**Kes**	**Nys**	**Tetra**	**Penta**	**Hexa**	**Hepta**	**Total**
Onion I											
0 months	35.2±0.9	6.2±0.3	2.2±0.3	68.1±5.3	75.0±1.9	54.5±1.5	103.6±7.7	96.5±5.6	90.7±4.6	120.9±10.1	602.0±35.0
6 months	25.0±0.2	5.3±0.2	2.0±0.0	59.1±1.4	63.5±0.0	50.8±0.2	103.0±7.3	92.7±2.1	90.9±9.4	108.1±2.4	552.7±4.2
12 months	29.9±0.2	6.4±0.3	2.4±0.4	66.7±5.9	68.4±5.6	58.3±1.9	100.5±10.0	97.8±8.8	88.6±7.9	109.7±9.6	573.4±36.0
Onion II											
0 months	18.5±1.7	6.4±0.4	1.5±0.1	50.2±5.3	49.2±4.4	46.0±3.5	100.9±10.0	110.5±11.8	102.3±10.6	124.2±11.6	565.9±52.3
6 months	20.0±0.1	5.7±0.5	2.0±0.0	39.1±0.9	55.8±2.5	51.6±3.7	95.4±9.2	101.1±9.2	100.2±9.0	110.4±5.3	532.6±0.7
12 months	23.1±1.1	6.1±0.3	2.1±0.2	49.5±4.8	55.1±2.8	57.3±1.7	97.1±8.8	99.9±3.3	95.2±4.1	121.8±3.1	552.4±9.8
Garlic											
0 months	2.0±0.0	-	2.7±0.1	19.5±0.6	7.0±0.7	6.0±0.5	7.6±0.2	4.5±0.4	5.4±0.5	-	48.5±2.3
6 months	1.5±0.1	-	2.2±0.0	21.1±0.1	7.4±0.2	7.1±0.6	7.6±0.7	5.4±0.5	5.0±0.6	-	50.1±0.4
12 months	1.8±0.2	-	2.3±0.0	22.0±0.1	7.1±0.6	6.6±0.4	6.8±0.1	5.8±0.6	5.2±0.4	-	51.0±1.4

*Content of carbohydrates is expressed per kilogram of dry matter. Values represent mean ± standard deviation, n = 2.

With respect to the storage at room temperature for 1 year of dehydrated vegetable samples, hardly any modification was produced in any of the carbohydrates analysed after 6 and 12 months of storage. This is in good agreement with the scarce evolution of MR in these samples, evidenced by the limited formation of 2-FM-AA (**Table 4.3**).

Regarding carrot samples, the evolution of MR was not enough to greatly affect the high reducing carbohydrate content of these samples. Similarly, Rahman et al. (2010) described no significant differences in total carbohydrate content of dehydrated carrots stored during 8 months at ambient temperature. In commercial powder onion and garlic samples stored under accelerated conditions (50 °C and 0.44 a_w), Cardelle-Cobas et al. (2009) reported important changes due to MR and hydrolysis in the carbohydrate fraction, including FOS. However, in the present paper, the milder storage conditions selected to simulate those generally employed in the market and by consumers, contributed to maintain the stability of these dehydrated products. Furthermore, the onion here analysed seems to be adequate as raw material for drying purposes, not only for its low content in monosaccharides, which contributes to a limited advance of MR, but also for its high content in prebiotic carbohydrates (FOS). This fact highlights the importance of selection of the most suitable cultivar, degree of maturity of raw product for the intended industrial processing.

With regard to *myo*-inositol, it can be observed that all samples contain this compound, carrot samples presenting the highest amount (4-6 g/kg DM) and potato samples the lowest (0.3 g/kg DM). Similar results were found in dehydrated carrots, onions and potatoes analysed by other authors (Clements & Darnell, 1980; Keller et al., 1998; Soria et al., 2009a; Hernández-Hernández et al., 2011). However, no data has been found in the literature for garlic. The presence of *myo*-inositol in foods is important because it might help to protect against cancer and other pathologies such as diabetes Mellitus and chronic renal failure (Clements & Darnell, 1980; Steinmetz & Potter, 1996).

Conclusions

According to the results obtained, it can be said that storage conditions assayed in the present paper, which are similar to those of the market and those used by consumers, have no appreciable effect on the quality parameters studied (dry matter, rehydration ability, total polyphenols, MR indicators, proteins and carbohydrates), probably due to the fact that the vegetables were properly treated and dehydrated at the industry. Therefore, from this point of view, it has been probed that these dehydrated carrot, potato, onion, and garlic are sufficiently stable for at least 12 months. These results are of particular relevance in the case of constituents with certain bioactivity. Moreover, the scarce MR advance also guarantees the preservation of nutritive value due to lysine. As nowadays there is an increasing interest in the use of dehydrated vegetables as food ingredients in the elaboration of a number of foodstuffs, the data here reported are quite valuable for technologists, nutritionists and consumers.

4.1.1.1.2. Evaluación de la calidad de frutas deshidratadas

Survey of quality indicators in commercial dehydrated fruits

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Journal of Food Composition and Analysis (submitted)

Abstract

Physical and chemical quality parameters (dry matter, a_w, protein, carbohydrates, vitamin C, 2-furoyl-methyl amino acids, rehydration ratio and leaching loss) have been determined in 30 commercial dehydrated fruits (strawberry, blueberry, raspberry, cranberry, cherry, apple, grapefruit, mango, kiwifruit, pineapple, melon, coconut, banana and papaya). For comparison purposes, two strawberries processed in the laboratory by freeze-drying and by convective drying were also used as control samples. Overall quality of dehydrated fruits seemed to be greatly dependent on processing conditions and, in a cluster analysis, samples which were presumably subjected to osmotic dehydration were separated from the rest of fruits. These samples presented the lowest concentration of vitamin C and the highest evolution of Maillard reaction, as evidenced by its high concentration of 2-furoyl-methyl amino acids. This is the first study on the usefulness of this combination of chemical and physical indicators to assess the overall quality of commercial dehydrated fruits.

Introduction

Fruits and vegetables of premium quality are currently highly appreciated by consumers, not only for their high nutritional value and pleasant organoleptic properties, but also for their content in bioactive compounds (vitamins and antioxidants, among others) directly related to health benefits (Szajdek & Borowska, 2009; Giampieri et al., 2012). Thus, several fruits like kiwifruit, papaya, strawberry, pineapple or grapefruit are

highly appreciated for their high content of vitamin C, a major natural antioxidant compound (Szajdek & Borowska, 2009; USDA, 2013).

Although fruits are usually consumed as fresh products, they are seasonal in nature and highly perishable and, therefore, they are frequently processed to obtain longer shelf-life products such as juice, fruit beverage, wine, jam, marmalade, jelly, frozen and dehydrated products, etc (De Ancos et al., 2000; Sanz et al., 2001; Rada-Mendoza et al., 2002). Among them, dehydrated products are gaining considerable attention due to the present life style and, in recent years, the presence of dehydrated fruits in the market has increased considerably. In addition to fulfill direct consumers' demand, large amounts of dehydrated fruit production are addressed for the industrial elaboration of breakfast cereals, bakery, desserts and confectionery products. In 2006, the European Union production of dehydrated fruits amounted to 1700 million euros corresponding to 428 thousand tons and their consumption was valued at 2300 million euros and 871 thousand tons. Italy, the United Kingdom and Spain were the three largest markets (CBI, 2008).

Although different dehydration processes are used by food processing industries, convective drying is the most common due to its simplicity of operation and affordable technology. Freeze-drying (FD), the best method of water removal to obtain final products of the highest quality, is also used in the industry (Krokida & Maroulis, 1997; Marques et al., 2009; Asami et al., 2003). However, its high energetic costs make this process only profitable for the dehydration of high-value products (Ratti, 2001).

Another important aspect to be considered in the dehydration of vegetables and fruits is the pre-treatment applied (Agnieszka & Andrzej, 2010a; Gamboa-Santos et al., 2013a). Osmotic dehydration (OD), in which the food is immersed in solutions of different sugars, is one the most common pre-treatments applied in industry and it provokes water loss and soluble solids exchange (Fernandes et al., 2011; Nahimana et al., 2011). These mass exchange processes might have an effect on the organoleptic properties and/or nutritional value of the dehydrated product, and may lead to final products with very different quality attributes (Lewicki, 2006).

Furthermore, during the whole dehydration process, important changes affecting the quality of the food can also be produced, and their extent depends on the conditions used. Thus, severe heating favors the loss of

thermolabile compounds such as vitamin C (Erle & Schubert, 2001; Frías et al., 2010a; Wojdylo et al., 2009). Moreover, this compound together with other soluble solids as sugars, acids, minerals, hydrophilic vitamins, etc. can also be lost by leaching during OD (Devic et al., 2010). Other important change is the Maillard reaction (MR) that can also occur during drying and storage of the final products. This reaction is influenced by factors such as water activity (a_w), temperature, pH and chemical composition of foods. In the first stage of MR, Amadori compounds are formed and their derivatives, the 2-furoyl-methyl amino acids (2-FM-AA), have been previously reported as useful markers of the evolution of this reaction in dehydrated vegetables and food products derived from fruits (Sanz et al., 2001; Rada-Mendoza et al., 2002; Rufián-Henares et al., 2008; Soria et al., 2009b; Wellner et al., 2011). The evaluation of the Amadori compounds provides very valuable information for process control and for nutritional evaluation, as it reveals not only the loss of available essential amino acids as lysine, but also of other amino acids such as arginine, whose content in fruits might be reduced by MR.

Shrinkage and hardening are the most important physical changes taking place during drying of dehydrated fruits. These are due to modification of tissue microstructure (Krokida & Maroulis, 1997) and to chemical changes affecting saccharides and proteins (Soria et al., 2010), and they can negatively affect the rehydration ability of dehydrated fruits (Lewicki, 2006; Sagar & Kumar, 2010).

The aim of the present study was to evaluate different chemical and physical quality indicators (humidity, a_w, protein, carbohydrates, vitamin C, 2-FM-AA, rehydration ratio (RR) and leaching loss (LL)) in 30 commercial dehydrated fruits, in order to determine their nutritional quality and to tentatively identify the kind of processing to which they have been subjected in the industry. For comparative purposes, these parameters were also determined in two additional samples processed in the laboratory by convective drying and by FD. To the best of our knowledge, this is the first study in which Maillard reaction (MR) together with the other chemical and physical indicators have been assessed in this sort of samples.

Materials and methods

Samples

Twelve samples of dehydrated strawberries (*Fragaria x ananassa*), two cranberries (*Vaccinium oxycoccos*), two blueberries (*Vaccinium corymbosum*), one raspberry (*Rubus idaeus*), two cherries (*Prunus avium*), two kiwifruits (*Actinidia chilensis*), two coconut (*Cocos nucifera*), one banana (*Musa sapientum*), one apple (*Pyrus malus*), one grapefruit (*Citrus paradisi*), one mango (*Mangifera indica*), one papaya (*Carica papaya*), one pineapple (*Ananas comusus*) and one melon (*Cucumis melo*) were purchased from local markets in Madrid and Barcelona (Spain) and in Fribourg (Germany). Seven of these samples were labeled as freeze-dried products and no information on the process was provided for the rest of fruit samples analyzed. In addition, raw strawberries were laboratory dehydrated using a convective prototype (7 h, 60 ºC, 4 m/s air rate; Gamboa-Santos et al., submitted; *section 4.1.1.2.2*) or a freeze-dryer and they were used as control samples. Dehydrated fruits were stored at a refrigeration temperature of 4 ºC up to one week before analysis.

Characterization of samples

The dry matter (DM) content of samples was gravimetrically determined in an oven at 102 ºC until constant weight according to the AOAC (1990a). Water activity (a_w) measurement was carried out in an AW Sprint TH-500 instrument (Novasina, Pfäffikon, Switzerland). Protein content was determined using the Kjeldahl method (AOAC, 1990b) using 6.25 as conversion factor.

Determination of carbohydrates

Carbohydrates were extracted from dehydrated fruits previously ground to powders using a laboratory mill IKA A-10 (IKA Labortechnik, Staufen, Germany). Thirty milligrams of sample were weighted into a polyethylene tube and extracted at room temperature with 2 mL of Milli-Q water under constant stirring for 20 min. Then, 8 mL of absolute ethanol were added,

followed by 0.2 mL of an ethanolic solution 10 mg/mL of phenyl-β-D-glucoside (Sigma-Aldrich Chemical, St. Louis, Missouri, USA) used as internal standard. After stirring for 10 min, samples were centrifuged at 9,600*g* for 10 min and the supernatant was collected. Precipitates were subjected to a second extraction with 10 mL of 80% ethanol under the same conditions to obtain recovery values close to 100%. Finally, an aliquot (2 mL) of merged supernatants was evaporated under vacuum at 40 °C and derivatized.

Trimethylsilyl oximes (TMSO) of saccharides were prepared according to Sanz et al. (2004). Oximes were obtained by adding 200 µL of a 2.5% solution of hydroxylamine hydrochloride in pyridine and heating the mixture at 70 °C for 30 min. These derivatives were then silylated with hexamethyldisilazane (200 mL) and trifluoroacetic acid (20 mL) at 50 °C for 30 min. Reaction mixtures were centrifuged at 7,000*g* for 2 min at room temperature. Supernatants were injected into the GC system or stored at 4 ºC prior to analysis.

Carbohydrate analyses were carried out following the method of Soria et al. (2010). Analyses were performed on an Agilent Technologies gas chromatograph (Mod 7890A) equipped with a flame ionization detector (GC-FID). Separation was carried out in a fused silica capillary column HP-5MS (25 m x 0.32 mm x 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Nitrogen at a flow rate of 1 mL/min was used as carrier gas. The oven temperature was held at 200 ºC for 11 min, raised to 315 ºC at a heating rate of 15 ºC/min and held for 5 min. Injector and detector temperatures were 280 and 315 ºC, respectively. Injections were made in split mode (1:30). Data acquisition and integration were done using Agilent ChemStation Rev. B.03.01 software (Wilmington, DE, USA).

For quantitation, standard solutions of glucose, fructose, *myo*-inositol, sucrose, kestose and mannitol over the expected concentration range in extracts of dehydrated fruits were prepared and analysed in triplicate to calculate the response factor relative to phenyl-β-D-glucoside. All determinations were carried out in duplicate and data were expressed as mean ± standard deviation (SD).

Analysis of 2-furoyl-methyl amino acids (2-FM-AA)

Determination of 2-FM-AA in dehydrated fruits was performed by ion-pair RP-HPLC following the method of Resmini & Pellegrino (1991). Before analysis, samples (250 mg) were hydrolyzed with 4 mL of 8 N HCl at 110 ºC for 23 h under inert conditions. The hydrolyzate was filtered through Whatman No. 40 filter paper and 0.5 mL of filtrate was applied to a previously activated (methanol and water) Sep-Pak C_{18} cartridge (Millipore). 2-FM-AA were eluted with 3 mL of 3 N HCl and 50 µL were injected into the chromatograph. RP-HPLC analysis was carried out in a C_8 column (250 mm x 4.6 mm, 5 µm) (Alltech furosine-dedicated, Nicolasville, KY) thermostatised at 37 ºC, using a linear binary gradient at a flow rate of 1.2 mL/min. Mobile phase consisted of solvent A, 0.4% acetic acid, and solvent B, 0.3% KCl in phase A. The elution program was as follows: 100% A from 0 to 12 min, 50% A from 20 to 22.5 min, and 100% A from 24.5 to 30 min. Detection was performed using a variable wavelength UV detector set at 280 nm (Beckman System 166, Fullerton, CA, USA). Acquisition and processing of data were achieved with Karat 8.0 Software (Beckman 140 Coulter Inc., Brea, CA, USA). Identification of 2-FM-Lys was done by using a commercial standard of pure furosine (Neosystem Laboratories, Strasbourg, France). Data reported on amino acid composition of fruits (Souci et al., 1987; Blanch et al., 2012; USDA, 2013) and on elution order of 2-FM-AA analysed under identical experimental conditions (Soria et al., 2009b) were also considered for tentative identification of 2-FM-Arg. Quantitation was performed by the external standard method, using furosine as standard. All analyses were done in duplicate, and data shown are the average value ± SD.

Determination of vitamin C

The procedure employed to determine total vitamin C (ascorbic acid plus dehydroascorbic acid) was the reduction of dehydroascorbic acid to ascorbic acid, using D,L-dithiothreitol as reducing reagent (Gamboa-Santos et al., 2013b). Total vitamin C content of dehydrated fruits was determined by liquid chromatography with diode array detection (RP-HPLC-DAD) on an Agilent Technologies 1220 Infinity LC System – 1260 DAD (Boeblingen, Germany). The separation of vitamin C was carried out with an ACE 5 C_{18}

column (ACE, UK) (250 mm x 4.6 mm, 5 µm) thermostated at 25 ºC. Elution was done under isocratic conditions (5 mM KH_2PO_4, pH 3.0) at a flow rate of 1 mL/min for 10 min. Injection volume was 20 µL and data acquisition and processing were performed using the Agilent ChemStation software (Agilent Technologies, Germany). Quantitation was performed by the external standard method, using a commercial standard of ascorbic acid (Sigma) in the range 0.3–50 mg/L.

Fruit extracts were prepared by adding 12.5 mL of 0.4% oxalic acid to 0.25 g of previously lyophilized samples and homogenizing for 1 min at 13500 rpm using an Ultra-Turrax T-25 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany). After addition of 2.5 mL of a 5 mg/mL solution of D,L-dithiothreitol, fruit extracts were kept at room temperature in the darkness for 30 min. Once the volume of the slurries was made up to 25 mL with Milli-Q water, they were centrifuged at 3200 *g* for 5 min. The supernatant was filtered through 0.45 µm syringe filters and further analysed by RP-HPLC-DAD. Fruit extracts were made in duplicate.

Rehydration ability

Rehydration of fruit samples at room temperature was performed according to Soria et al. (2010), using 1:50 as solid-to-liquid ratio. Rehydration ratio (RR) was calculated as:

$$RR = m_r/m_d$$

where m_r is the mass of the rehydrated sample (g) and m_d is the weight (g) of the dehydrated fruit.

Loss of soluble solids during rehydration was gravimetrically determined. The soak water was evaporated until constant weight. The residue was weighed, and the percentage of leached solids (LL, %) with respect to the initial weight of dehydrated fruit was calculated. All determinations were carried out in duplicate.

Statistical analysis

Data for all physical and chemical quality indicators in the 32 dehydrated fruits under study were subjected to cluster analysis (linkage: Ward's method, distance measure: 1-pearson r) to determine similar groups of accessions.

Results and discussion

Characterization of samples

Table 4.6 shows the percentages of dry matter and protein and the a_w values determined in the different dehydrated fruits under analysis. As it can be seen, the DM content ranged from 76.2 to 94.3% and the a_w values from 0.216 to 0.561. On the basis of a_w values, and irrespective of the fruit considered, two grouping of samples might be inferred: Group-I, including commercial freeze-dried samples and strawberry-D1, with a_w values up to 0.292; and Group-II formed by the rest of dried fruits, with $a_w \geq 0.360$.
Control samples showed the lowest a_w, with values of 0.142 and 0.208 for freeze-dried and convective-dried strawberries, respectively. In general, both DM and a_w values were low enough to guarantee the microbiological stability of dehydrated products. It has been described that pathogenic bacteria do not grow in media with a_w values lower than 0.85, whereas molds and yeasts are more tolerant (a_w values as low as 0.80, Sagar et al., 2010).

Although no microbiological growth has been reported to occur in dehydrated vegetables and fruits at a_w values lower than 0.62 (Sagar et al., 2010), other modifications as non-enzymatic browning is only avoid at a_w below 0.3 (Belitz et al., 2009a; Corzo-Martínez et al., 2012). According to Moraga et al. (2012), a_w values lower than 0.210 can slow down the deteriorative reactions of bioactive compounds (organic acids, vitamin C, main flavonoids, and total phenols) during the storage of grapefruit powder. Among the dehydrated fruits analysed in this paper and, despite some of them were very close to this value, only the control strawberry samples presented values of a_w lower than 0.210.

Table 4.6 Data on DM, a_w and protein determined in the dehydrated fruit samples under study (data shown as average ± SD).

Dehydrated Fruit	Sample code	DM (%)	a_w	Protein (%)[1]
Strawberry-FD[2]1	1	82.9 ± 0.4	0.251 ± 0.007	5.3 ± 0.3
Strawberry-FD2	2	81.5 ± 0.1	0.245 ± 0.004	6.1 ± 0.6
Strawberry-FD3	3	84.8 ± 0.0	0.229 ± 0.027	6.9 ± 0.3
Strawberry-FD4	4	76.4 ± 2.0	0.280 ± 0.010	5.3 ± 0.4
Strawberry-D1	5	77.0 ± 1.1	0.292 ± 0.028	4.9 ± 0.5
Strawberry-D2	6	87.7 ± 0.8	0.476 ± 0.023	0.4 ± 0.1
Strawberry-D3	7	84.0 ± 1.0	0.424 ± 0.016	0.3 ± 0.0
Strawberry-D4	8	82.4 ± 0.2	0.514 ± 0.006	0.7 ± 0.1
Strawberry-D5	9	81.8 ± 0.6	0.492 ± 0.005	0.6 ± 0.0
Strawberry-D6	10	89.1 ± 0.0	0.423 ± 0.042	0.6 ± 0.2
Strawberry-D7	11	87.1 ± 0.1	0.404 ± 0.011	0.5 ± 0.1
Strawberry-D8	12	78.1 ± 0.8	0.479 ± 0.014	0.5 ± 0.0
Blueberry-FD	13	86.5 ± 0.8	0.216 ± 0.015	3.7 ± 0.2
Blueberry-D	14	80.2 ± 0.9	0.458 ± 0.017	1.2 ± 0.1
Raspberry-FD	15	87.2 ± 1.0	0.267 ± 0.023	8.8 ± 0.3
Cranberry-D1	16	79.6 ± 0.5	0.526 ± 0.007	0.4 ± 0.0
Cranberry-D2	17	76.2 ± 0.1	0.523 ± 0.006	0.4 ± 0.0
Cherry-FD	18	78.6 ± 1.5	0.281 ± 0.002	8.6 ± 0.3
Cherry-D	19	77.5 ± 0.2	0.494 ± 0.028	0.6 ± 0.0
Apple	20	82.6 ± 0.8	0.513 ± 0.018	0.2 ± 0.0
Grapefruit	21	88.8 ± 0.5	0.414 ± 0.015	0.3 ± 0.0
Mango	22	78.9 ± 0.2	0.557 ± 0.062	0.3 ± 0.0
Kiwifruit-D1	23	80.1 ± 0.3	0.497 ± 0.035	0.5 ± 0.0
Kiwifruit-D2	24	87.0 ± 0.0	0.514 ± 0.010	0.4 ± 0.0
Pineapple	25	89.6 ± 0.3	0.532 ± 0.015	0.1 ± 0.0
Melon	26	85.9 ± 0.2	0.360 ± 0.030	n.d.[3]
Coconut-D1	27	89.0 ± 0.0	0.561 ± 0.008	1.3 ± 0.2
Coconut-D2	28	94.3 ± 1.0	0.486 ± 0.013	7.1 ± 0.4
Banana	29	93.2 ± 0.7	0.508 ± 0.009	2.0 ± 0.0
Papaya	30	86.3 ± 0.6	0.560 ± 0.013	0.1 ± 0.0
Strawberry-FD-Lab	31	82.0 ± 1.2	0.142 ± 0.013	6.2 ± 0.5
Strawberry-D-Lab	32	82.0 ± 0.7	0.208 ± 0.004	6.5 ± 0.3
Mean		**86.1 ± 5.9**	**0.524 ± 0.053**	**2.6 ± 3.1**

[1] %: g/100 g DM
[2] FD: freeze-dried; D: dehydrated (unknown procedure)
[3] n.d.: not detected

As a result of the different samples considered and their probably diverse processing conditions, the protein content of dehydrated fruits (**Table 4.6**) varied widely (0.1-8.8%). In this case, a similar trend to that shown for a_w was observed: samples included in Group-I showed high protein contents (above 3.7%), while the protein percentage of samples in Group-II was lower than 2.0% and a large number of them (18 samples) contained less than 1.0% of protein. This might be due to the fact that most of fruits in Group-II could have been subjected to OD pre-treatments. During OD, the water loss gives rise to a gradual breakdown of the tissue due to pectin solubilisation, causing a loss of shape of cellular walls and turgor pressure (Prinzivalli et al., 2006). This, together with the high osmotic pressure, might provoke solute uptake and leaching, increasing the concentration of solutes present in the osmotic solution and the dilution of other compounds, such as proteins.

Analysis of carbohydrates

Table 4.7 lists the results of the carbohydrate analysis of the dehydrated fruits under study. Glucose, fructose and sucrose were the major sugars; their concentrations showed a wide variability as expected for the different samples considered: 244 ± 110 g/kg DM, 238 ± 96 g/kg DM and 206 ± 153 g/kg DM, respectively. Five dehydrated fruits could be considered as particular cases: blueberry-FD, blueberry-D and cherry-FD with very low sucrose content (≤ 3 g/kg DM), and coconut-D2 and banana with glucose and fructose values lower than 16 g/kg DM.

Table 4.7 Data on carbohydrates (g/kg DM) and glucose/fructose and sucrose/glucose ratios calculated for the dehydrated fruits analysed

Dehydrated fruit	Total sugars (%)[1]	g/kg DM (average ± SD)						Glu/Fru	Suc/Glu
		Glucose (Glu)	Fructose (Fru)	Sucrose (Suc)	Mannitol	*Myo*-Inositol	Kestose		
Strawberry-FD[2]1	44.5 ± 2.4	155 ± 7	184 ± 12	107 ± 8	n.d.[3]	2.4 ± 0.2	0.7 ± 0.1	0.8	0.7
Strawberry-FD2	59.3 ± 3.2	249 ± 10	291 ± 14	52 ± 8	n.d.	3.3 ± 0.6	1.1 ± 0.2	0.9	0.2
Strawberry-FD3	65.4 ± 4.1	240 ± 21	280 ± 25	133 ± 6	n.d.	2.9 ± 0.1	0.8 ± 0.1	0.9	0.6
Strawberry-FD4	64.3 ± 1.1	297 ± 1	313 ± 49	32 ± 3	n.d.	2.0 ± 0.4	1.4 ± 0.3	0.9	0.6
Strawberry-D1	62.7 ± 3.6	210 ± 15	255 ± 17	161 ± 10	n.d.	6.5 ± 1.3	0.9 ± 0.4	0.8	0.8
Strawberry-D2	80.2 ± 4.6	228 ± 20	216 ± 17	354 ± 22	50.8 ± 2.0	n.d.	5.1 ± 0.8	1.1	1.6
Strawberry-D3	76.7 ± 3.7	251 ± 14	236 ± 15	276 ± 10	56.6 ± 4.6	n.d.	3.6 ± 0.3	1.1	1.1
Strawberry-D4	85.4 ± 0.9	368 ± 7	345 ± 11	130 ± 12	n.d.	n.d.	11.1 ± 1.0	1.1	0.4
Strawberry-D5	88.1 ± 1.0	229 ± 9	220 ± 9	422 ± 6	n.d.	0.6 ± 0.1	10.9 ± 0.8	1.0	1.8
Strawberry-D6	73.7 ± 3.8	246 ± 22	226 ± 19	258 ± 15	57.8 ± 5.5	n.d.	7.2 ± 0.7	1.1	1.0
Strawberry-D7	82.0 ± 2.5	323 ± 11	296 ± 10	185 ± 3	24.2 ± 2.0	n.d.	15.2 ± 1.2	1.1	0.6
Strawberry-D8	91.2 ± 3.4	263 ± 21	258 ± 25	388 ± 38	n.d.	n.d.	3.1 ± 0.4	1.0	1.5
Blueberry-FD	69.1 ± 1.1	335 ± 3	354 ± 8	3 ± 0	n.d.	0.8 ± 0.2	n.d.	0.9	0.0
Blueberry-D	80.1 ± 3.6	455 ± 22	345 ± 14	n.d.	n.d.	0.6 ± 0.1	n.d.	1.3	-
Raspberry-FD	26.5 ± 1.7	77 ± 6	95 ± 8	87 ± 4	1.1 ± 0.3	0.7 ± 0.3	2.5 ± 0.2	0.8	1.1
Cranberry-D1	83.6 ± 4.8	413 ± 29	363 ± 14	58 ± 5	n.d.	n.d.	2.1 ± 0.2	1.1	0.1
Cranberry-D2	80.9 ± 4.1	377 ± 20	348 ± 19	76 ± 4	n.d.	n.d.	8.4 ± 0.7	1.1	0.2
Cherry-FD	62.9 ± 0.8	343 ± 5	257 ± 24	2 ± 0	78.2 ± 8.0	6.5 ± 1.0	8.0 ± 1.0	1.3	0.0
Cherry-D	91.5 ± 4.6	394 ± 18	368 ± 35	149 ± 12	n.d.	n.d.	4.2 ± 0.4	1.1	0.4
Apple	88.3 ± 1.2	296 ± 17	289 ± 12	286 ± 16	n.d	n.d.	13.4 ± 1.4	1.0	1.0
Grapefruit	81.4 ± 1.8	170 ± 3	170 ± 5	463 ± 12	13.2 ± 0.4	n.d.	11.4 ± 1.0	1.0	2.7
Mango	86.1 ± 1.2	313 ± 5	289 ± 8	243 ± 24	n.d.	n.d.	15.1 ± 0.7	1.1	0.8
Kiwifruit-D1	81.2 ± 4.8	259 ± 20	257 ± 18	287 ± 19	n.d.	n.d.	8.6 ± 0.6	1.0	1.1
Kiwifruit-D2	75.5 ± 2.6	145 ± 1	141 ± 0	460 ± 25	2.2 ± 0.6	n.d.	8.5 ± 0.9	1.0	3.2
Pineapple	76.8 ± 0.9	168 ± 1	164 ± 1	426 ± 11	2.9 ± 0.7	n.d.	10.0 ± 0.6	1.0	2.6
Melon	86.6 ± 3.1	324 ± 27	300 ± 24	224 ± 18	n.d.	n.d.	18.0 ± 2.0	1.1	0.7
Coconut-D1	52.0 ± 1.1	92 ± 7	84 ± 7	335 ± 25	n.d.	n.d.	8.9 ± 0.6	1.1	3.7
Coconut-D2	12.8 ± 0.2	4 ± 0	4 ± 0	110 ± 1	4.3 ± 1.6	8.5 ± 1.5	1.1 ± 0.4	1.0	27.5
Banana	19.0 ± 0.8	13 ± 1	16 ± 2	156 ± 10	4.6 ± 0.4	n.d.	6.2 ± 1.0	0.8	12.0
Papaya	86.3 ± 0.6	161 ± 4	158 ± 1	542 ± 9	40.3 ± 2.6	n.d.	2.9 ± 0.4	1.0	3.4
Strawberry-FD-Lab	54.9 ± 5.0	212 ± 19	243 ± 22	95 ± 12	n.d.	4.1 ± 0.2	0.9 ± 0.1	0.9	0.4
Strawberry-D-Lab	54.1 ± 3.5	210 ± 19	240 ± 22	93 ± 12	n.d.	3.9 ± 0.3	0.8 ± 0.2	0.9	0.4
Mean	**41.5 ± 32.0**	**244 ± 110**	**238 ± 96**	**206 ± 153**	**10.5 ± 21.4**	**1.4 ± 2.3**	**5.9 ± 5.2**	**1.0 ± 0.1**	**2.3 ± 5.1**

[1]%: g/100 g DM; [2]FD: freeze-dried; D: dehydrated (unknown procedure); [3]n.d.: not detected

Considering the content of individual carbohydrates, it might be assumed that some of the samples analyzed could have been treated with different osmotic solutions. Thus, and according to Muir et al. (2009) and data of USDA (2013), raw apple and mango fruits show an excess of fructose (ratio Glu/Fru 0.4-0.6 and 0.4-0.7, respectively) which is not evidenced in the ratios Glu/Fru here calculated. This suggests that a glucose solution might have been used for the OD of these fruits. Similarly, and according to the Suc/Glu ratio of fresh fruits (USDA, 2013), a wide number of samples (strawberry-D2, D3, D5, D6 and D8, cherry-D, grapefruit, kiwifruit-D1 and D2, pineapple, banana, papaya) might have been treated with a sucrose solution.

Regarding total carbohydrate content, Heng et al. (1990) estimated that, after osmotic treatment, papaya samples gained a 42% of sugar relative to the DM. In agreement with this, the total sugar content of most of samples of Group-II was very high and, in eighteen of them, total carbohydrates were higher than 70% referred to DM, value that exceeds the corresponding taking into account the fresh fruit (USDA, 2013). With respect to the other samples, three of them presented a concentration of total sugars lower than 30% (coconut-D2, banana and raspberry-FD). These were in agreement with sugar content of fresh fruit, except for banana whose carbohydrate content in fresh mature fruits is noticeably higher (49%, USDA, 2013). This points out to the fact that this fruit could have been harvested unripe when the fruit had high starch and low soluble sugar levels (Adao et al., 2005).

With respect to minor carbohydrates, kestose (a prebiotic sugar) was quantified in all the samples except for the two blueberries. However, the content was very low, with an average value of 5.9 g/kg DM. Mannitol was present in twelve of the samples studied and its concentration was relatively high (13.2-78.2 g/kg DM) only in strawberries-D2, D3, D6 and D7, cherry-FD, grapefruit and papaya. The content of this polyalcohol, which can be naturally present in many fruits, has been described to be particularly high in cherries, its content varying widely (2.2-8.0 g/100 g of fresh weight) depending on the variety (Girard & Kopp, 1998). The fact that this compound was not detected in one of the cherry samples analysed could be due to its loss during OD. Contrarily, and according to data reported in the literature

(Makinen & Soderling, 1980), no natural presence of mannitol has been previously described in ripe strawberry; therefore, the presence of this polyalcohol in some of the dried strawberries here studied might be indicative of a pre-treatment by OD with a solution of mannitol. With respect to papaya and grapefruit samples, to the best of our knowledge, the presence of mannitol has not previously been reported. Therefore, the origin of this compound is uncertain and OD pre-treatment should not be discarded. Agnieszka & Andrzej (2010b) found that OD pre-treatment with mannitol gives rise to an upsurge in mechanical resistance and an increase of compression work for dry material. As positive effects, mannitol confers sweetness and decreases the glycemic index, resulting in food products more suitable for diabetics. EFSA (2010) has recently recognised that mannitol induces a lower blood glucose rise after their consumption as compared to other foods containing sugars and contributes to the maintenance of tooth mineralisation.

Other minor compound such as *myo*-inositol, presents in variable concentrations depending on the dehydrated fruit considered (Clements, 1980; Sanz et al., 2004), can also be lost during the process of OD. This would explain the scarce content of this compound in most of the dehydrated fruits here analysed.

Determination of vitamin C

The retention of vitamin C is often used as an estimation of the overall nutritional quality of fruits and vegetables (Goula & Adamopoulos, 2006). As shown in **Table 4.8**, control samples processed in the laboratory showed the highest amount of vitamin C, with values of 5610 and 4329 mg/kg DM for freeze-dried and convective-dried strawberry samples. As compared to fresh strawberries, these values represent 100 and 77% of vitamin retention, respectively.

Table 4.8 Determination of vitamin C and 2-FM-Lys + 2-FM-Arg in dehydrated fruit samples analysed

Dehydrated Fruit	vitamin C (mg/kg DM)	2-FM-Lys + 2-FM-Arg (mg/kg protein)
Strawberry-FD1	2487 ± 238	2676 ± 203
Strawberry-FD2	2460 ± 20	1476 ± 145
Strawberry-FD3	3650 ± 24	464 ± 5
Strawberry-FD4	221 ± 5	4750 ± 405
Strawberry-D1	647 ± 64	4209 ± 177
Strawberry-D2	n.d.[1]	7151 ± 690
Strawberry-D3	n.d.	7834 ± 738
Strawberry-D4	n.d.	4881 ± 36
Strawberry-D5	n.d.	6080 ± 589
Strawberry-D6	n.d.	3253 ± 161
Strawberry-D7	n.d.	9822 ± 562
Strawberry-D8	n.d.	1971 ± 189
Blueberry-FD	873 ± 57	448 ± 13
Blueberry-D	n.d.	10437 ± 223
Raspberry-FD	1016 ± 94	1444 ± 63
Cranberry-D1	2 ± 0	6090 ± 595
Cranberry-D2	6 ± 0	1371 ± 75
Cherry-FD	15 ± 1	384 ± 23
Cherry-D	4 ± 0	2318 ± 70
Apple	3 ± 0	13471 ± 190
Grapefruit	n.d	8987 ± 351
Mango	n.d.	17793 ± 190
Kiwifruit-D1	2 ± 0	3780 ± 235
Kiwifruit-D2	n.d.	5278 ± 161
Pineapple	n.d.	14609 ± 1141
Melon	n.d.	-[2]
Coconut-D1	n.d.	10477 ± 1040
Coconut-D2	n.d.	2842 ± 281
Banana	n.d.	1646 ± 71
Papaya	n.d.	37663 ± 2552
Strawberry-FD-Lab	5610 ± 450	n.d.[3]
Strawberry-D-Lab	4329 ± 345	1996 ± 52
Mean	**666 ± 1435**	**6309 ± 7378**

[1]n.d: not detected (detection limit: 1 mg/kg DM)
[2]Protein not detected; in this sample 2-FM-Lys + 2-FM-Arg was 9 ± 1 mg/kg product
[3]n.d: not detected

The amount of vitamin C was also remarkable for most freeze-dried samples, with contents higher than 221 mg/kg DM. The low content of vitamin C determined in cherry-FD (15 mg/kg DM) could be explained by the ten-fold lower amount of this vitamin detected in fresh cherry as compared to

that of fresh strawberry (Girard & Kopp, 1998). The remaining samples showed a content of this vitamin very low (2-6 mg/kg DM) and, in many cases (n=17), it was not even detected.

Vitamin C is a very sensitive indicator whose loss in dehydrated fruits can be attributed to osmotic treatment, dehydration and storage conditions, among other factors. Several authors have observed a high loss of vitamin C by leaching during OD of different fruits (Taiwo et al., 2001; Azoubel et al., 2009; Devic et al., 2010). The effect of temperature during OD on the loss of vitamin C was also studied in papaya and kiwifruit by Vial et al. (1991). At 40 °C, a decrease of only 30% was measured after 3.5 h, whereas at 50 °C losses up to 90 % were determined. Wojdylo et al. (2009), in a study on drying of strawberry at 70 ºC for 550 min, reported a vitamin C loss of 72%, and similar results were obtained by Frías et al. (2010a) in carrots dried at 65 ºC for 6 h. Borquez et al. (2010), in raspberries osmotically pre-treated and microwave-dried, reported a vitamin C loss of 80%, and half of these losses occurred during drying due to chemical deterioration by heat. Peñas et al. (2012) reported a drastic decrease of this vitamin in dehydrated vegetables stored at ambient temperature for 12 months. The degradation of vitamin C during storage may be attributed to browning reactions by spontaneous thermal decomposition under both aerobic and anaerobic conditions (Namiki, 1988).

Comparing the results of commercial strawberries with those of control samples obtained in the laboratory under controlled conditions and not subjected to storage, the concentration of vitamin C in the latter was considerably higher, particularly for the freeze-dried control sample. It is well established that the retention of vitamin C in freeze-dried products is significantly higher than that of oven and sun-dried products (Santos & Silva, 2008; Sagar & Kumar, 2010).

Assessment of initial stages of Maillard reaction

Although furosine is a well-recognized indicator in other dried vegetables and fruits, this was the first time that 2-FM-AA were detected in these dehydrated fruits. As an example, **Figure 4.4** shows the chromatograms obtained during the HPLC analysis of the acid hydrolysates of coconut-D1 and

strawberry-D4. **Table 4.8** lists the 2-FM-Lys + 2-FM-Arg values determined in all the dehydrated fruits under analysis. In a previous study, Sanz et al. (2001), for commercial dried samples of raisins, prunes, figs, dates, and apricots obtained values of 2-FM-Lys + 2-FM-Arg between 77 at 625 mg/kg product, respectively. These levels were higher than the obtained in this paper (4-193 mg/kg product corresponding to 384-37663 mg/kg protein).

As in previous determinations, dehydrated fruits seemed to be divided into two classes according to their 2-FM-AA content. Thus, samples in Group-I were characterized by a low 2-FM-Lys + 2-FM-Arg content, ranging from 384 to 4750 mg/kg protein, whereas the rest of samples analyzed (Group-II) showed levels in the range 1371 to 37663 mg/kg. The mild dehydration conditions could justify the lower level of this quality indicator in fruits of Group-I, most of them processed by FD. However, it is well known that during storage a significant loss of nutrients occurs in dried fruits and vegetables and this loss is dependent on storage temperature, pH, exposure to oxygen, porosity, light and presence of organic acids (Sagar & Kumar, 2010). The effect of storage, among others, could explain that sample strawberry-FD4 had a higher level of 2-FM-AA and lower of vitamin C than the dried strawberry-D1.

Although both proteins and free amino acids can participate in the MR, in dehydrated fruits presumably subjected to OD, amino acids are thought to be easily lost by leaching, protein being the main source of amino compounds to be involved in the MR. Thus, the protein damage due to MR in samples of Group-II seems to be more severe. Regarding control samples, furosine was not detected in the freeze-dried strawberry, and its content in convective-dried strawberry was 1996 mg/kg of protein, very close to the lowest value of commercial samples. Among others, the process and storage conditions (temperature, time, etc) to which samples have been subjected might explain the differences observed in the 2-FM-AA content of commercial as compared to laboratory processed samples.

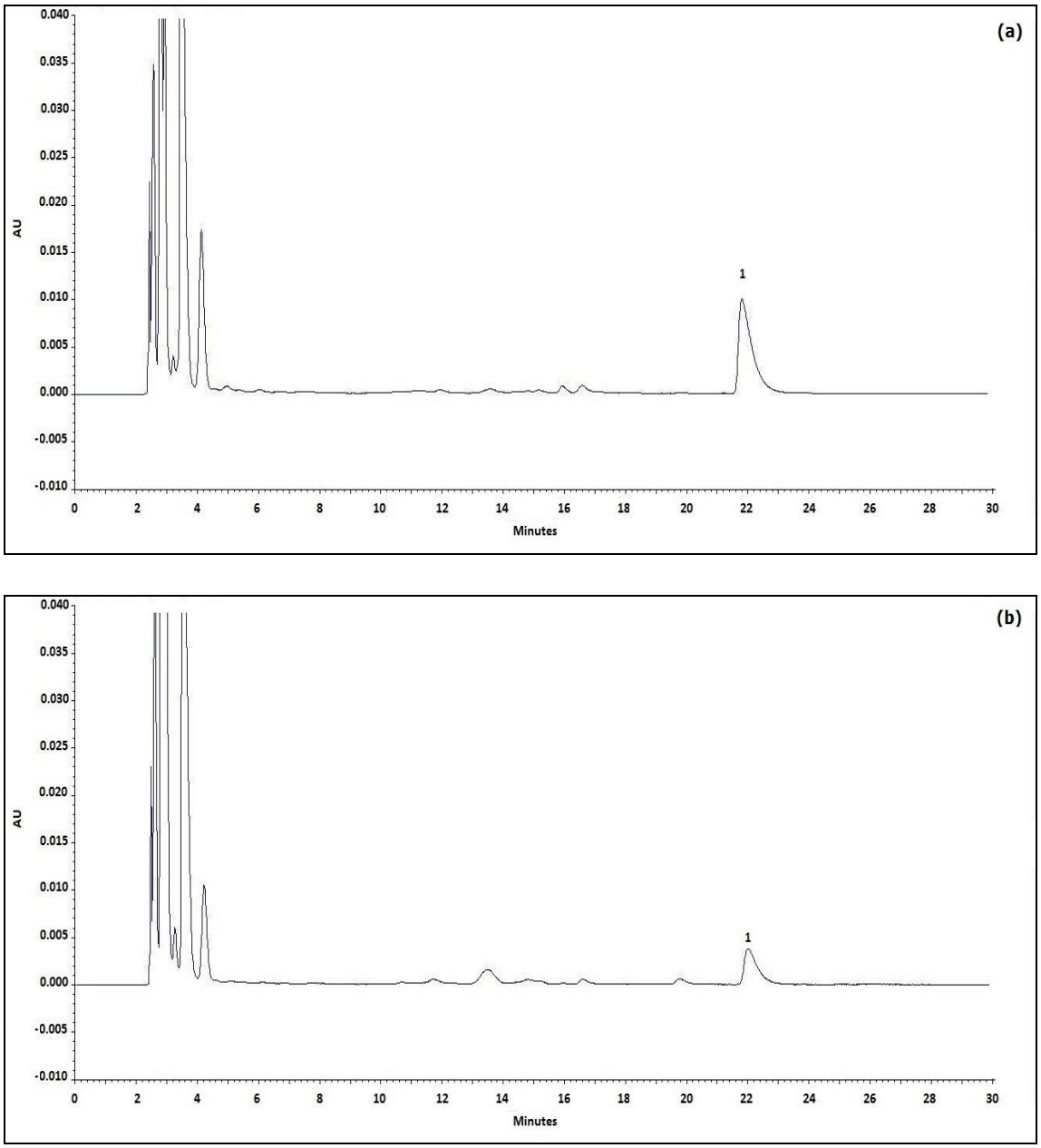

Figure 4.4 HPLC chromatogram of 2-furoyl-methyl amino acids in acid hydrolysate of sample coconut-D1 (a) and strawberry-D4 (b). Peak 1: 2-FM-Lys + 2-FM-Arg.

Rehydration ability

Rehydration ability (RR) and leaching losses (LL) are two physical parameters that can provide valuable information about the final quality of dehydrated products. As observed in **Table 4.9**, the range of RR in commercial samples was 1.0-6.9. In this case, as in previous determinations, two groups of samples could be distinguished: Group-I–with values from 3 to 6, and Group-II, with values, in general, below 2.2. An exception of Group-II

was the dehydrated apple that presented a RR value of 3.6. Krokida & Marinos-Kouris (2003) observed that dried apple presented RR values higher than those of other fruits and vegetables dehydrated under the same conditions.

In general, the freeze-dried samples (commercial and laboratory processed) had the highest RR. These results are in agreement with those reported by Marques et al. (2009) and by Agnieszka & Andrzej (2010a). On the contrary, OD samples presented very low RR values. This could probably be due to the substantial changes affecting the properties of the material, including the softening of the tissue, that take place during OD carried out with high concentration of sugars (Lewicki, 2006). Agnieszka & Andrzej (2010a) observed a decrease in the rehydration capacity of the freeze-dried strawberries osmotically dehydrated in sucrose or glucose solutions, with respect to no-pre-treated samples. Moreover, the low RR value of some of the dehydrated samples of the present study could also be explained by the film of oil or wax coating the fruit, as is the case of blueberry-D, whose ingredient information included vegetable oil.

In relation to the values of LL, it can be observed that, in general, a great solid loss was produced (705 ± 150 g/kg DM), and only in three samples (raspberry-FD, banana and coconut-D1) the loss was less than 50% of DM. Maldonado et al. (2010) also reported a solid loss of 71% during rehydration of dried mango osmotically pre-treated. These results highlight that rehydration should be done in the food in which the fruit is going to be used as ingredient (yoghourt, cake, etc) and not previously in water. Alternatively, dehydrated fruits might be directly (without rehydration) consumed as snacks. Furthermore, certain individual differences in RR and LL might be attributable to the different sample geometry, as in coconut-D1 and D2 (sliced and diced, respectively).

Table 4.9 Data (average ± SD) on rehydration ratio (RR) and leaching loss (LL) of the dehydrated fruits analysed

Dehydrated fruit	RR	LL (g/kg DM)
Strawberry-FD1	4.8 ± 0.4	691 ± 51
Strawberry-FD2	4.3 ± 0.4	724 ± 12
Strawberry-FD3	6.9 ± 0.0	617 ± 40
Strawberry-FD4	4.8 ± 0.4	597 ± 21
Strawberry-D1	3.0 ± 0.0	664 ± 21
Strawberry-D2	1.6 ± 0.1	762 ± 53
Strawberry-D3	2.0 ± 0.2	593 ± 41
Strawberry-D4	1.4 ± 0.0	756 ± 64
Strawberry-D5	1.8 ± 0.1	894 ± 26
Strawberry-D6	1.6 ± 0.1	909 ± 77
Strawberry-D7	2.1 ± 0.1	884 ± 7
Strawberry-D8	2.2 ± 0.1	882 ± 81
Blueberry-FD	3.4 ± 0.2	730 ± 1
Blueberry-D	1.0 ± 0.1	696 ± 35
Raspberry-FD	3.5 ± 0.2	463 ± 33
Cranberry-D1	1.3 ± 0.1	805 ± 5
Cranberry-D2	1.2 ± 0.1	794 ± 50
Cherry-FD	3.1 ± 0.3	804 ± 31
Cherry-D	1.5 ± 0.0	849 ± 46
Apple	3.6 ± 0.1	546 ± 53
Grapefruit	1.0 ± 0.1	870 ± 25
Mango	1.2 ± 0.1	841 ± 82
Kiwifruit-D1	1.5 ± 0.0	738 ± 05
Kiwifruit-D2	1.3 ± 0.0	761 ± 12
Pineapple	1.0 ± 0.1	873 ± 62
Melon	1.9 ± 0.1	710 ± 72
Coconut-D1	1.3 ± 0.0	381 ± 38
Coconut-D2	1.8 ± 0.1	682 ± 67
Banana	1.8 ± 0.2	374 ± 15
Papaya	1.0 ± 0.1	592 ± 20
Strawberry-FD-Lab	7.3 ± 0.4	649 ± 47
Strawberry-Lab	4.6 ± 0.3	440 ± 35
Mean	**1.5 ± 0.7**	**705 ± 150**

In order to explore the natural grouping of samples, data for all the quality parameters here analyzed were subjected to Cluster Analysis. As can be seen in **Figure 4.5**, with the exception of three outliers (strawberry-FD4, strawberry-D1 and cherry-FD), dehydrated fruit samples were classified into two groups, in agreement with the results previously indicated for individual

determinations. In this classification, the quality parameters with greater weight were the protein content (r = 0.792), rehydration ratio (r = 0.769) and a_w (r = -0.848). As result, irrespective of the fruit considered, Group-II seems to include all the samples that had been subjected to OD, whereas Group-I is mainly formed by freeze-dried samples.

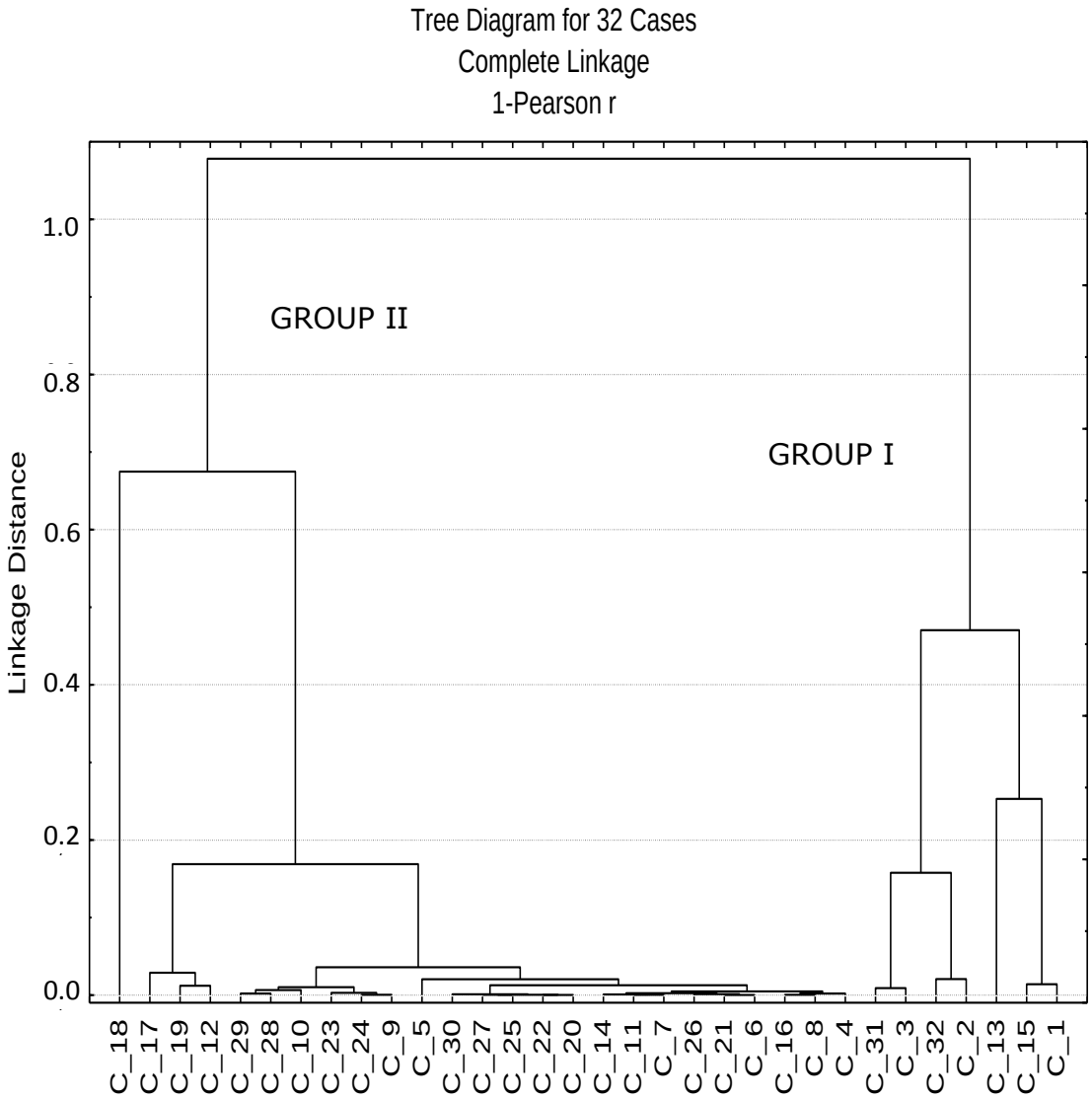

Figure 4.5 Cluster analysis of commercial and laboratory-dehydrated fruits. For identification of samples, see column 2 of Table 4.6.

Conclusions

According to the results obtained in this study, most of the dehydrated fruits marketed are probably produced by OD before convective drying. Although the organoleptic acceptance by consumers of OD fruits might be high (Azoubel et al., 2009), their overall quality is poor, particularly if the nutritional value is considered, as demonstrated by their low content of vitamin C and high of 2-FM-Lys + 2-FM-Arg (indicators of lysine and arginine loss).

It is also worth noting that solute uptake and leaching of valuable constituents (natural sugars, acids, minerals, vitamins) by OD often lead to a substantial modification of the original product composition and, for instance, osmotically dehydrated fruits are high calorie products. The so called "healthy snacking", consisting of a mixture of nuts and tropical dried fruits, is mainly targeted at young people, for whom obesity is an increasing health problem. For this reason, it is very important to control the dehydration process, including pre-treatment, so that products with scarce added sugar and minimally modified bioactive compounds are obtained. Since the current trends in production are addressed to fulfill this requirement (Giampieri et al., 2012), the results here reported on quality indicators are of great usefulness for food companies interested in the processing of dehydrated fruits with premium quality.

4.1.1.2 Deshidratación en un prototipo por convección

4.1.1.2.1 Optimización de las condiciones de deshidratación mediante Superficie de respuesta múltiple

Optimization of convective drying of carrots using selected processing and quality indicators

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International Journal of Food Science and Technology (2012), doi: 10.1111/ijfd.12076

Abstract

The effect of drying temperature (40-65 °C) and air rate (2-6 m s^{-1}) on the formation of Maillard reaction indicators and vitamins content of carrots dehydrated by convection has been investigated. The range of assayed processing conditions, based on a previous central composite face design, led to moderate changes in the studied parameters, even under the most severe conditions. In addition, the drying kinetic of the process was studied taking into account the experimental quantitation of shrinkage, which allowed the determination of a first drying period with a constant rate of water evaporation per unit of exchange surface. The slope of the first drying period, the moisture loss during the first hour of drying and the level of quality parameters (Maillard reaction indicators and vitamins) were correlated with processing conditions with high accuracy. For the prototype here used, the optima temperature and air rate to maximize the desirability function (0.77) were 46 °C and 4.9 m s^{-1}.

Introduction

Nowadays, the trends in food technology are addressed to the intake of nutritive and appealing foods which provide some health benefits to the consumers. Due to the present "style of life", dried foods and, particularly

vegetables, have a predominant position in the market of many countries and this is expected to increase even more over the next decade (Zhang et al., 2006). Longer shelf-life, product diversity and volume reduction are the main reasons for the popularity of dried vegetables (Lewicki, 2006).

Carrot (*Daucus carota* L.) constitutes an important vegetable for human nutrition due to its high vitamin, fibre and other valuable nutrients content and to its organoleptic properties. It is used fresh or dehydrated in the elaboration of a number of foodstuffs such as soups, salads, sauces, prepared meals and snacks. The importance of carrot is reflected by its global production which was estimated at 27 million metric tons in 2004 (Brunke, 2006).

Dried vegetables are mainly obtained by hot air drying (Lewicki, 2006), being temperature, air-flow rate and sample thickness the main parameters affecting the characteristics of the final product (Doymaz, 2004b). This process may cause irreversible chemical, physical and sensorial changes. At the low water activity and temperature conditions reached during drying, the Maillard reaction (MR) which involves reducing carbohydrates and free amino groups of amino acids, peptides, and proteins, can take place (Cardelle-Cobas et al*.,* 2005). Thus, 2-furoylmethyl-amino acids (2-FM-AA), formed at the early stages of MR, have been suggested as sensitive indicators in several dehydrated vegetables (Cardelle-Cobas et al., 2005; Sanz et al., 2001). These indicators have also been found in carrots submitted to different drying processes such as industrial and laboratory convective-drying (Rufián-Henares et al*.,* 2008; Soria et al., 2009b; Wellner et al., 2011) and ultrasound-assisted convective drying (Soria et al*.,* 2010). In this concern, particularly interesting is the case of the formation of 2-furoylmethyl-lysine (furosine) since its early detection can prevent advanced stages of the MR in which important losses of nutritive value due to the participation of lysine are produced (Corzo-Martínez et al., 2012). Other irreversible changes in the dried product can be related to vitamin losses and modification of texture, rehydration capability, flavour, colour and appearance (Lewicki, 2006).

During the last years, due to the increased consumer's awareness for better quality, safety and nutritional value of foods, drying research has been addressed toward the improvement of existent and/or emergent processing technologies which give rise to final products with improved characteristics.

One of the most common approaches to guarantee optimal quality of the final product is through careful process design. Thus, by means of tools such as modelization and optimization of the process, the efficiency of the drying can be improved. A number of studies related to the modelization of drying kinetics of carrot using convective dryers (Mulet, 1994; Singh & Gupta, 2007; Zielinska & Markowski, 2010), semi-industrial continuous convective dryers (Aghbashlo et al., 2009), or convective dryers assisted by ultrasound (Carcel et al., 2011; García-Pérez et al., 2007) have been conducted. In those studies, the selection of optimal conditions to obtain premium quality products is carried out by taking into account the interaction of selected processing parameters. In this sense, response surface methodology (RSM) is widely recognized as an important tool for process and product improvement. RSM enables to determine the relationship between the experimental factors (experimental drying variables) that simultaneously optimize the analysis variables (quality parameters) and maximize the desirability function (Myers et al., 2004). In several recent publications, RSM has been used for different drying procedures of artichoke and soybean (Icier, 2010), potato (Eren & Kaymak-Ertekin, 2007) and berries (Mitra & Meda, 2009). In carrots, RSM has been used for the optimization of osmotic dehydration (Kargozari et al., 2010; Singh et al., 2010; Sutar & Prasad, 2011) and fluidized bed processes (Mudahar et al., 1989; Nazghelichi et al., 2011). Aghbashlo et al. (2011) analysed the variation in the kinetic of carrot drying with the independent variables time, air temperature, air velocity, and cube size. Finally, Frías et al. (2010a) used RSM to study the effect of convective air drying of carrot on vitamin C and β-carotene retention; however, in that paper the drying kinetics was not studied. Moreover, to the best of our knowledge, no previous data have been reported in the literature on the optimization of convective drying of carrots based on 2-FM-AA data, as sensitive quality parameters.

In this paper, the effect of processing conditions (drying temperature and air rate) of a prototype by convection on quality indicators (Maillard reaction indicators and vitamins) and drying kinetic of sliced carrot have been reported. To this aim, an experimental design using a central composite face design (CCD) was first carried out and, then, the selected drying and quality parameters were related by a RSM in order to find the optima processing conditions leading to dehydrated carrots of the best quality.

Materials and methods

Samples

Fresh carrots (*Daucus carota* L. var. Nantesa) were purchased from a local market in Madrid (Spain). The selection of carrots was based on similar size, optimum colour and ripeness stage. After sorting, they were stored at 4 ºC for, as maximum, 5 days. Before processing, carrots were washed in tap water to remove dust and other residues and were peeled and sliced (4.0 ± 0.5 mm thickness and 24.0 ± 0.4 mm diameter). Sliced carrot samples were blanched in boiling water for 1 min (sample:water ratio was 1:12), cooled to room temperature in cold water and then dried with tissue paper to remove superficial water.

Drying equipment

Blanched carrot samples were dried by convection using a computer controlled (Edibon Scada Control and Data Acquisition Software) air tray dryer (SBANC, Edibon Technical Teaching Units, Spain; **Figure 4.6**). This system consists of three main sections: (i) fan unit with air rate control (AVE), (ii) temperature control (seven temperature sensors: ST1, ST4 and ST6 (dry bulb); ST2, ST5 and ST7 (wet bulb) and ST3 sensor of electrical resistance (AR)), and (iii) drying compartment (load cell with four drying trays). Although AR (ºC) was the setpoint temperature, ST7 (ºC) was chosen as representative of the process temperature since it was the wet bulb measurement closest to the sample. The air flow was parallel to the sample and the air rate was selected with the AVE (m s^{-1}) sensor. Experimental air-flow rate (m^3 h^{-1}) was verified at the output nozzle (area = 0.01 m^2) with a thermo-anemometer (TESTO, 425, Lenzkirch, Germany). During the drying process, the weight of the samples was automatically monitorized by the load cell of the system (SF, **Figure 4.6**). In addition, carrot samples were weighted at 1 h intervals using an external digital balance for control of accuracy of data (SOEHNLE, Murrhardt, Germany).

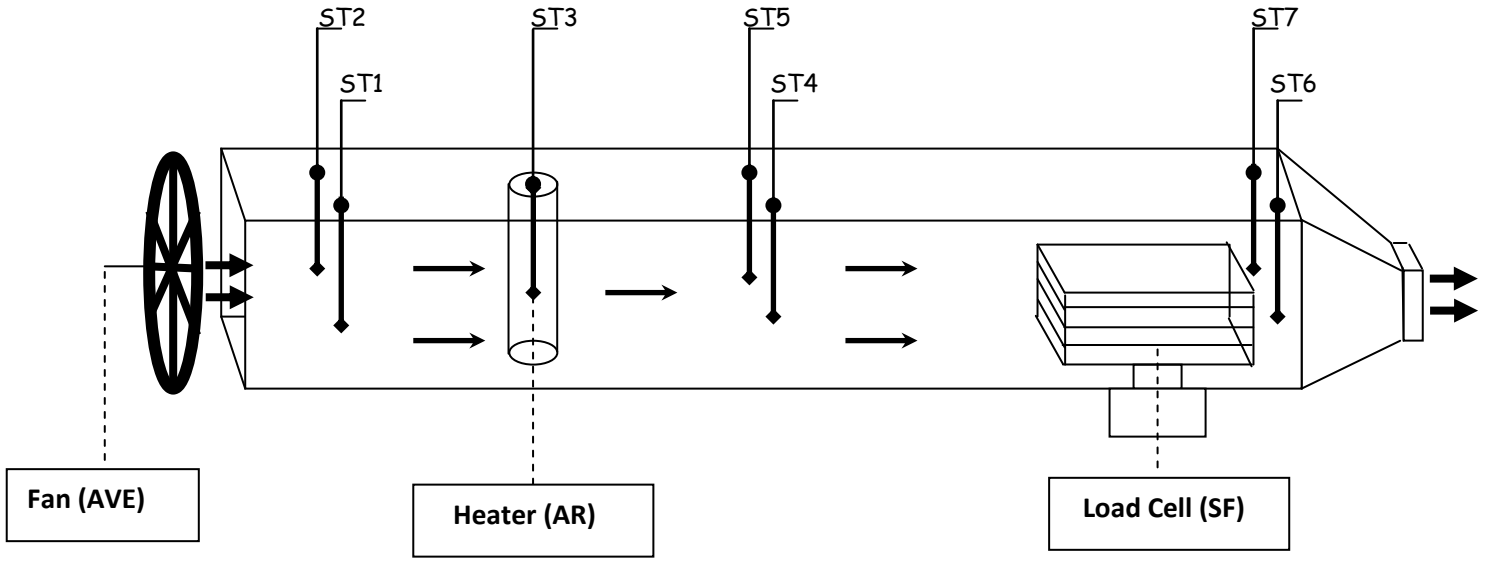

Figure 4.6 Drying cabinet (EDIBON) used for the convective drying of carrots.

Moisture content and drying kinetic curves

The kinetic curves representing the variation in the moisture content of carrots with convective drying time were calculated as follows:

$$X(t) = (W(t)-DM)/DM \quad (1)$$

where X is the moisture content (kg H_2O kg^{-1} DM) determined according to Geankoplis (1998), t is the drying time (h), W_t is the sample weight (kg) and DM the dry matter (kg) determined according to AOAC method (1990a).

A polynomial approximation (n=3) to the drying curve was proposed to fit the drying process. In agreement with Górnicki & Kaleta (2007), the first part of the drying curve was described by applying a linear regression model, assuming that a constant drying rate occurred during the first stage of process. Then, the drying rate decreased during the second or fall drying rate period. In the present paper, the end of the first constant drying period was considered by means of parameter t^*, defined as the period of time for which a linear regression of the drying curve can be attained with a $R^2 \geq 0.99$. In this point, the sample reached the critical moisture content (X^*). The slope (S) (kg H_2O kg^{-1} DM min^{-1}) of this trend line was also considered in the studied model.

Determination of shrinkage

To analyse the influence of shrinkage in the drying rate of the process, the thickness (l) and diameter (d) of carrots subjected to drying, were measured in triplicate (slices were selected at random) at 30 min-intervals using a vernier caliper (Mitutoyo Corp., Japan) (error ± 0.05 mm). Then, the real exchange surface area (A) was calculated as follows:

$$A = \pi d\,(d/2 + l) \quad (2)$$

In the previous equation, it is assumed that the cylindrical shape of carrots is maintained throughout the drying process; this assumption was supported by experimental observation of carrot slices, until the very last part of the falling rate drying period.

The water flux (q_t) averaged over the exchange surface area of carrot slice was calculated as previously defined by May & Perré (2002):

$$q_t = -\frac{DM}{A}\frac{dX(t)}{dt} \quad (3)$$

where $dX(t)/dt$ is the drying rate (kg H_2O kg^{-1} DM min^{-1}), which was calculated from the experimental drying curves.

Analysis of 2-furoylmethyl-amino acids

For determination of 2-FM-AA, 0.25 g of carrots were hydrolysed with 4 mL of HCl 8 M for 23 h at 110 ºC under inert atmosphere (helium), using a Pyrex screw-cap vial with polytetrafluoroethylene-faced septa (Soria et al., 2010). The resulting hydrolysate was filtered (paper filter Whatman no. 40) and 0.5 mL were purified in a Sep-Pack C_{18} cartridge (Millipore, MA) pre-treated with 5 mL of methanol and 10 mL of water. The filtrate was eluted with 3 mL of 3 M HCl.

The 2-FM-AA corresponding to lysine (furosine) and arginine were determined by ion-pair Reversed-phase-High-performance liquid chromatography (RP-HPLC) (Resmini & Pellegrino, 1991) using a C_8 column (250 mm length x 4.6 mm internal diameter, Alltech, Lexington, KY) at 37 ºC. A binary gradient composed of phase A (4 mL L^{-1} acetic acid) and phase B (3 g L^{-1} KCl in phase A solution) was used. The elution program was as follows: 0-12 min: 100% A; 20-22.5 min: 50% A and 50% B; 24.5-30 min: 100% A. The flow rate was 1.2 mL min^{-1} and injection (50 µL) was carried out using a manual Rheodyne valve. Detection was done at 280 nm in a LCD Analytical SM 4000 detector.

Quantification was performed by the external standard method, using a commercial standard of furosine (Neosystem Laboratoire, Strasbourg, France). Values were expressed as mg kg^{-1} protein and all the analyses were performed in duplicate.

To analyse the protein content of carrot samples under study, total nitrogen (TN) was determined by the Kjeldahl method (AOAC, 1990b). Protein content was calculated using 6.25 as conversion factor (TN x 6.25).

Optimization of carrot drying by response surface methodology

The effect of two independent factors, air rate and temperature, on the convective drying of blanched sliced carrots (80 g) for up to 6 hours was studied using a central composite face design (CCD, Statgraphic 5.0, Statistical Graphics Corporation, Rockville, MD, USA). A total of 10 experiments (2^2 points of a factorial design, 4 star points and 2 centre points to estimate the experimental error), were carried out in randomized order. Setpoint parameters, AR and AVE (**Figure 4.6**), of assays selected from the experimental design are listed in **Table 4.10**.

Four dependent variables were taken into account to optimize the convective drying of carrot by means of RSM: linear time (t^*, min), the slope of the linear function of the constant drying rate period (S, kg H_2O kg^{-1} DM min^{-1}), the weight loss at the first hour of processing (W_1, %) and the content of 2-FM-Lys + 2-FM-Arg (mg kg^{-1} protein). In addition, the level of vitamin C and β-carotene, determined in these samples by Frías et al. (2010a), were also included in the process optimization. Each analytical response was evaluated with a one-way analysis of variance (ANOVA) by using Fisher's Significant Difference test (LSD, 95%) (Statgraphics Centurion XV, Statistical Graphics Corporation, Rockville, MD, USA).

Table 4.10 Assay conditions (prototype setpoint and experimentally measured) of the experimental design for optimization of convective drying of carrot

	Prototype setpoint		**Experimental data**	
Assay	Temperature (AR, ºC)	Air rate (AVE, m s^{-1})	Temperature (ST7, ºC)	Air flow-rate ± standard deviation (m^3 h^{-1})
A1	62	2.0	52.5	67.3 ± 12.4
A2	50	5.4	43.6	198.8 ± 12.5
A3	64	4.0	52.5	154.3 ± 12.5
A4	53	2.6	43.6	93.0 ± 8.8
A5[a]	>80	5.4	61.4	198.8 ± 12.5
A6	64	4.0	52.5	154.3 ± 12.5
A7	80	4.0	65.0	154.3 ± 12.5
A8	72	6.0	52.5	218.8 ± 11.4
A9	72	2.6	61.4	93.0 ± 8.8
A10	43	4.0	40.0	154.3 ± 12.5

[a]Conditions excluded from experimental design as they were unfeasible to be obtained with the prototype used for convective drying.
AR, electrical resistance; AVE, air rate control of fan unit; ST7, temperature of wet bulb closest to the sample (± 0.5 ºC accuracy)

The analysis was based on the F-test and on the percentage of explained variance (R^2_{adj}), which provides a measurement of how much of the variability in the observed response values could be explained by the experimental factors and their interactions (Myers et al., 2004). The overall effect of the six dependent factors was used to obtain a desirability function that represents the effect of the processing conditions on the final product quality and on the efficiency of drying. It is based on the idea that the "quality" of a process has multiple quality characteristics (Reis et al., 2008). The method finds operating conditions that provide the "most desirable" response values. For processing conditions, a desirability function assigns numbers between 0 (completely undesirable value) and 1 (completely desirable or ideal response). To obtain process optimized, the models that presented an adjusted determination coefficient (R^2_{adj}) ≥ 70% were subjected to simultaneous optimization, in accordance with the procedures outlined by Granato et al. (2010).

Results and discussion

Drying kinetic

Table 4.10 lists the experimental conditions (ST7 and air-flow rate) corresponding to setpoint values (AR and AVE) taken from experimental design. Assay 5 (AR>80 ºC and AVE=5.4 m s^{-1}) was removed from the experimental design since the proposed combination of processing conditions was unfeasible to be accomplished by using prototype described under "Drying equipment" subsection. ST7 registered temperatures from 40 to 65 ºC that were obtained by setting AR at temperatures within the range 43-80 ºC. A close match was found between AVE values (2-6 m s^{-1}) and experimental air rate data (in the range 1.9-6.1 m s^{-1}) calculated from air-flow rate measured by using a thermoanemometer. Blanched carrot samples (with an initial DM of 10.5%) processed under the operating conditions listed in **Table 4.10** showed percentages of DM between 84.1 and 89.2. These values are close to those considered as appropriate to preserve the microbiological quality of dehydrated vegetables (~85%, Belitz at al., 2009a). These values corresponded to an initial moisture content of 9.65 ± 0.34 kg

H_2O kg^{-1} DM for blanched carrot samples and, after drying, the moisture content was in the range 0.44- 0.99 kg H_2O kg^{-1} DM.

For each of the assays listed in **Table 4.10** (except for assay A5), variation in the moisture content of the sample as a function of time was calculated from data collected at 1 h intervals for up to 6 hours. **Figure 4.7** depicts the experimental measurements and the corresponding polynomial fit. As expected, the moisture decreased with drying time for all drying processes resulting in different curves depending on the processing conditions of each assay.

In general, as it has been described in the literature (Geankoplis, 1998), both the constant rate period and the falling-rate period, described in the drying of solids under constant conditions, were experimentally observed. In the initial period, a vegetable like carrot with high moisture content shows a constant rate of drying. This is due to the fact that evaporation initially takes place near the surface, and water is easily transported to the surface by diffusion. Therefore, the rate of drying would be the same than the rate of free water evaporation. In such conditions, the interface temperature remains constant and the heat is completely used for water evaporation (Geankoplis, 1998). In the second, falling-rate period, the decrease of drying rate might be related to the reduction in porosity of the material due to shrinkage, with the progress of drying increasing the resistance to movement of water (Lagunez-Rivera et al., 2007; Singh & Gupta, 2007). In this period, the diffusion of internal moisture to the solid surface is the rate-limiting step, when compared with the rate at which the surface moisture is swept away; therefore it is the period of diffusion-controlled drying (Cárcel et al., 2007b).

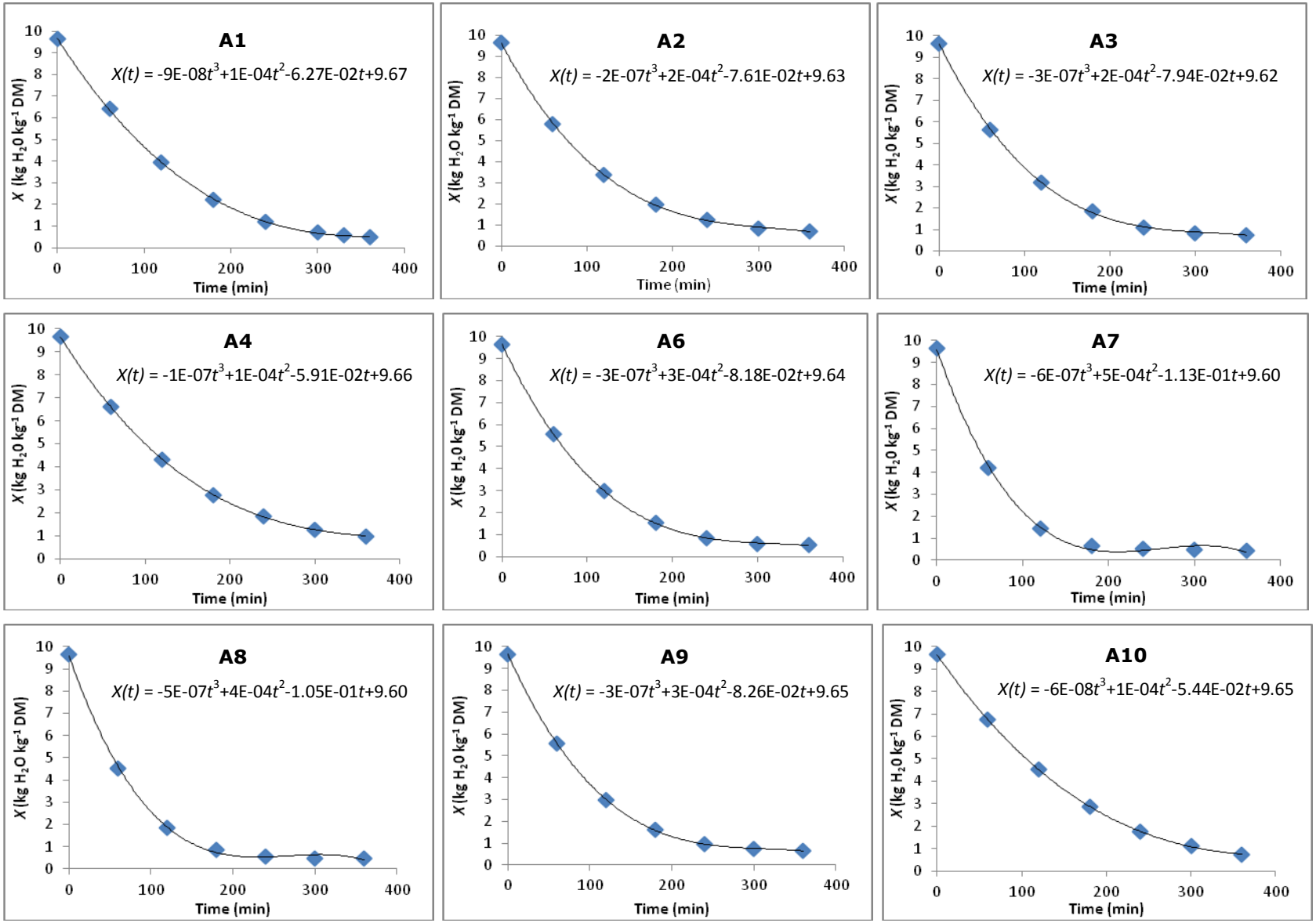

Figure 4.7 Drying curves at different air-flow rates and temperatures (Table 4.10) for carrot samples (R^2>0.99).

Different papers report fruit or vegetable (including carrot) dehydration, with these two periods (Dissa et al., 2008; Saravacos & Charm, 1962). However, many studies consider that there is no stage of constant rate or it has been assumed that the first period is negligible because of the changes in water content are not linear after a short period from the beginning of drying; therefore, the entire drying process is considered to occur in the range of falling-rate period (Arslan & Mehmet, 2010; Doymaz, 2008b; García-Pérez et al., 2007; Mulet, 1994). These contradictory reported results could be related, among other factors, to the importance of considering the shrinkage and shape changes during the first period of drying. Thus, during the constant rate period, the shrinkage can be neglected and the conditions of external mass transfer could determine the course of the process (Górnicki & Kaleta, 2007). However, May & Perré (2002) and Pabis & Jaros (2002) stated that in the case of foods with high initial moisture content, the kinetic model must incorporate the shrinkage factor for better describing the drying results.

In order to analyse the effect of shrinkage on the drying process, **Figure 4.8** shows, as an example, the water flux (q_t) as a function of the drying time under the conditions of centre point assays (A3, A6), considering not only the slice surface equal to the initial surface but also the actual slice surface reported in **Table 4.11**. As can be deduced from the figure, a constant rate period was observed when shape changes (reduction of slice surface) were considered.

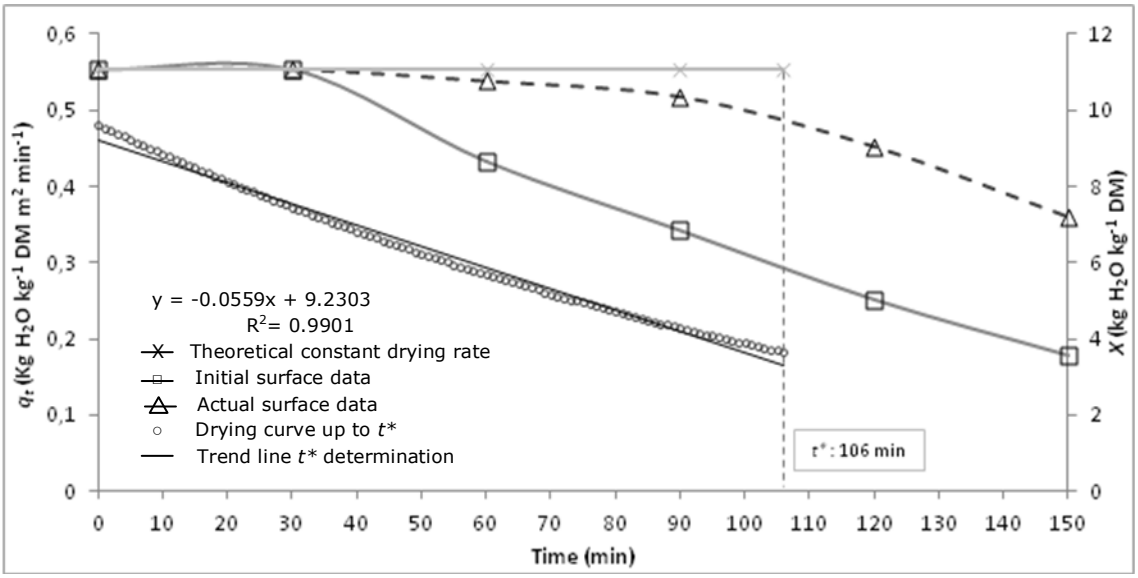

Figure 4.8 Water flux (q_t) and moisture content (X) *vs.* time for the first stage of drying curve at the centre point.

Table 4.11 Experimental thickness (l) and diameter (d) (average data ± SD) used to study the influence of shrinkage during drying of carrots under A3 conditions

Time (min)	*d (mm)*	*l (mm)*
0	23.2 ± 0.4	4.0 ± 0.5
30	21.0 ± 0.4	3.4 ± 0.3
60	19.1 ± 0.4	3.0 ± 0.3
90	17.8 ± 0.6	2.4 ± 0.3
120	16.8 ± 0.4	2.3 ± 0.2
150	15.1 ± 0.8	2.1 ± 0.3
180	13.4 ± 0.7	2.0 ± 0.1
210	12.0 ± 1.2	1.8 ± 0.4
240	12.1 ± 1.7	1.4 ± 0.4
270	12.4 ± 1.7	1.8 ± 0.6
300	11.9 ± 1.4	1.8 ± 0.2
330	11.8 ± 1.3	1.9 ± 0.3
360	12.4 ± 1.0	2.0 ± 0.7

The first section of drying curve of A3 assay is also represented in **Figure 4.8**, together with the t^* parameter calculated as the time for which a linear regression of the drying curve can be obtained with a precision of R^2 = 0.99. As can be observed, the value of this parameter reasonably corresponds to the constant rate period observed. Then, the t^* parameter for each assay of carrot drying was calculated (see **Table 4.12**) and was included as a dependent variable in the model analysis. The highest values of t^* were found in samples processed under the conditions of assays A1, A4 and A10 (148, 141 and 192 min, respectively), which were the mildest of the

experimental design; whereas the lowest values of *t** were detected in the most intense assays (A7, 81 min; A8, 85 min). Taking into account the *t** drying time, the remaining critical moisture content (*X**) was calculated (**Table 4.12**), and ranged from 2.4 to 3.9 kg H_2O kg^{-1} DM depending on processing conditions, values similar to those reported by May & Perré (2002) for convective drying of carrots (2.8 kg H_2O kg^{-1} DM). According to Dissa et al. (2008), the value of this remaining moisture can be considered as the critical moisture for each drying process since it separates both constant and falling rate periods.

Taking into account the *t** definition, the drying rate during the constant rate period can be calculated for each experimental assay as the slope (*S*) of the linear regression obtained. The *S* values obtained are given in **Table 4.12** and are in agreement with the *t** values, namely the higher *t**, the lower value for the slope, that is the lower drying rate. The highest slopes of the drying curves were obtained for assays A7 and A8 (0.080 and 0.074 kg H_2O kg^{-1} DM min^{-1}, respectively), corresponding to the most severe conditions. Several authors have reported that, in general, drying rates increase with the temperature and air-flow rate for various vegetables including carrot (Velic et al., 2004; Aghbashlo et al., 2009; Zielinska & Markowski, 2010). However, when the air rate range is narrow (0.5 - 1 m s^{-1}), hardly any effect on drying rate can be detected (Madamba et al., 1996).

Table 4.12 Values of *t** (linear time), *X** (critical moisture content), *S* (constant rate period of drying), W_1 (weight loss at the first hour of processing), and concentration of 2-FM-AA (average ± SD) at the end of the processing of carrot samples

Assay	*t** (min)	*X** (kg H_2O kg^{-1} DM)	*S* (kg H_2O kg^{-1} DM min^{-1})	W_1 (%)	2-FM-Lys + 2-FM-Arg (mg kg^{-1} protein)
A1	148	3.5	-0.044	30.5	393 ± 23
A2	106	3.9	-0.054	36.2	tr[a]
A3	106	3.7	-0.056	37.8	445 ± 19
A4	141	3.8	-0.042	28.5	tr
A5	--	-	--	--	--
A6	106	3.3	-0.058	37.8	431 ± 1
A7	81	3.2	-0.080	50.9	1689 ± 152
A8	85	3.2	-0.074	48.1	1583 ± 20
A9	105	3.5	-0.058	38.3	705 ± 35
A10	192	2.4	-0.038	27.2	tr

[a]tr: trace value. For RSM optimization, trace values were replaced by an arbitrary numeric value of 0.1.

During the constant rate drying period, values between 27 and 51% of weight loss were reached after 1 hour of drying (**Table 4.12**). The highest W_1 values were observed in the assays carried out under the most severe conditions (A7 and A8), whereas the lowest values for this parameter (about 28%) were obtained at mild processing conditions (A4, and A10).

Effect of drying on quality indicators

As afore-mentioned, the formation of 2-FM-Lys + 2-FM-Arg was selected as quality marker of drying process related to the initial steps of evolution of MR. **Table 4.12** depicts the quantitative data of 2-FM-AA for carrots analysed in the present work after 6 h of dehydration. No formation of 2-FM-AA was detected either in raw or blanched carrots. The highest 2-FM-AA contents (1689 and 1583 mg kg^{-1} protein) were found in dehydrated carrots after the assays A7 and A8, respectively. The conditions used in these assays were the most severe and also gave rise to the major humidity losses (residual humidity values of 10.8 and 12.7%). The intermediate processing conditions (A1, A3, A6 and A9) provoked the formation of 2-FM-AA within the range 393-705 mg kg^{-1} protein, and only traces of 2-FM-AA were detected in carrot samples processed after assays A2, A4 and A10.

The 2-FM-Lys (furosine) contents of dehydrated carrots analysed in the present work were lower than those previously reported for dehydrated carrots by other authors. Soria et al. (2009b; 2010) found values from 3580 to 8480 mg kg^{-1} protein in industrially dried and commercial carrot samples, whereas upper values were obtained by Wellner et al. (2011) for commercial carrot products (15408-15529 mg kg^{-1} protein) and for carrot slices dried at 70, 80 and 90 °C in an oven during 5 h (9040 - 9890 mg kg^{-1} protein). Rufián-Henares et al. (2008) analysed carrots industrially dehydrated at low temperature (30 ºC) during long time (180 h) and they found values of 4030 mg furosine kg^{-1} protein. Only in carrots dehydrated by power ultrasound at temperatures up to 60 ºC, Soria et al. (2010) reported values of this quality marker significantly lower than those here analysed (390 mg kg^{-1} protein).
These results highlight the limited progress of MR during the dehydration process of carrot carried out in our convective system, even under the most

severe conditions of temperature and air flow, and the importance of optimizing the process to obtain premium quality products, since kinetic of this reaction is strongly dependent on temperature and water content throughout the treatment.

Optimization of processing conditions by response surface methodology

In order to optimize the drying process, operating conditions (air rate and drying temperature) of assays 1-10 were related by means of RSM with each of the dependent variables under study: parameters derived from drying curves (t^*, S and W_1) and quality parameters (2-FM-AA, vitamin C and β-carotene). The equations of the fitted models and the corresponding estimated responses surfaces are shown in **Table 4.13** and **Figure 4.9**, respectively.

Together with the equations of the fitted models given in **Table 4.13**, the R^2 and R^2_{adj} statistics values are also shown. As it can be observed, with the exception of t^*-value, all the variables presented high values of R^2 and R^2_{adj} indicating the goodness of the fits (Granato et al., 2010). The slopes of the constant rate period present high regression values (R^2 = 98.9% and R^2_{adj} = 97.1%), showing that this variable can be maximized in the optimization by RSM, in order to attain the shorter times during the drying process. Consequently, the slope and weight loss at the first hour (R^2 = 99.5% and R^2_{adj} = 98.7%) of each fit were selected as the representative variables of the drying process together with the quality indicators of dried carrot samples, defined as the 2-FM-Lys + 2-FM-Arg content (measured and reported in this work) and the vitamin C and β-carotene contents.

Table 4.13 Regression equations for the model fit of the different variables studied during the drying process of carrot

Variables**	Fitted Model Equation	R^2 (%)	R^2_{adj} (%)
t^* (min)	$656.645–15.973 \cdot T–7.231 \cdot V+0.125 \cdot T^2–0.127 \cdot T \cdot V–0.0974 \cdot V^2$	81.8	51.5
S (kg H_2O kg^{-1} DM min^{-1})	$0.088-0.002 \cdot T-0.018 \cdot V+1.863E-05 \cdot T^2+3.871E-04 \cdot T \cdot V+7.169E-04 \cdot V^2$	98.9	97.1
W_1 (%)	$52.161-1.022 \cdot T-10.072 \cdot V+0.0103 \cdot T^2+0.210 \cdot T \cdot V+0.443 \cdot V^2$	99.5	98.7
2-FM-AA (mg kg^{-1} protein)	$997.268-28.035 \cdot T-239.366 \cdot V+0.215 \cdot T^2+3.303 \cdot T \cdot V+11.920 \cdot V^2$	97.0	92.1
Vitamin C (mg kg^{-1} DM)	$2.582+0.585 \cdot T+3.622 \cdot V-0.005 \cdot T^2-0.085 \cdot T \cdot V+0.053 \cdot V^2$	99.4	98.3
ß-carotene (mg kg^{-1} DM)	$8.169+1.097 \cdot T+5.657 \cdot V-0.012 \cdot T^2-0.030 \cdot T \cdot V-0.669 \cdot V^2$	98.6	96.4

** t^*, linear time; S constant rate period of drying, W_1 weight loss at the first hour of processing, 2-FM-AA, 2-furoylmethyl-amino acids

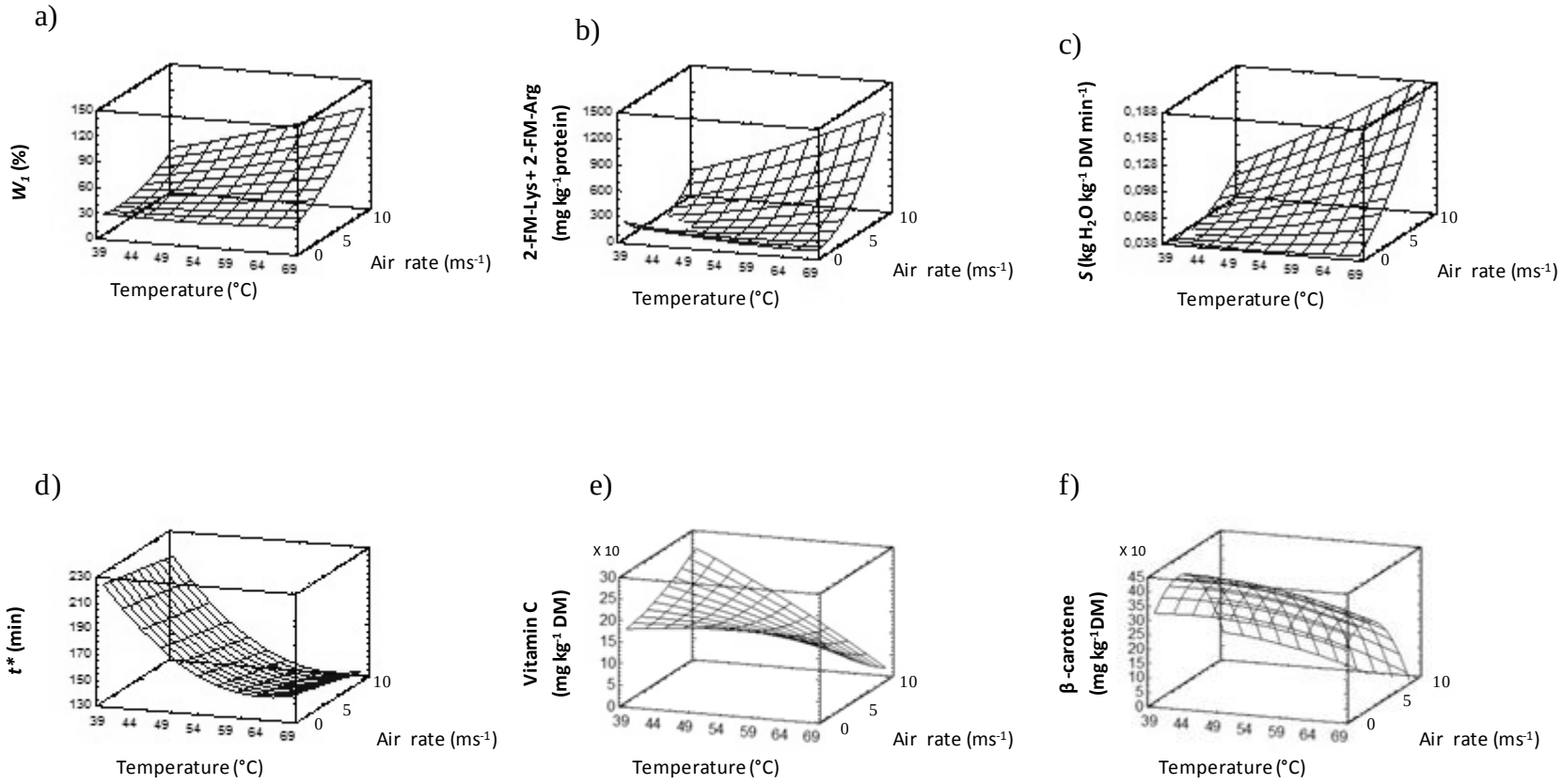

Figure 4.9 Response surface plots of each analysed variable as a function of temperature an air-flow rate.

Additionally, **Table 4.14** compares the value of the desirability function predicted for assays A1 to A10, with the values calculated from the corresponding experimental data. This was defined to minimize the 2-FM-AA concentration and maximize the β-carotene and vitamin C content, the first hour loss weight (W_1) and the rate of the first drying period (S).

Table 4.14 Predicted and observed values for the desirability function during the different assays of drying of carrots by convection

Assay	*Desirability*	
	Predicted	Observed
A1	0.61	0.61
A2	0.74	0.78
A3	0.73	0.73
A4	0.51	0.58
A5	-	-
A6	0.73	0.73
A7	0.00	0.00
A8	0.40	0.34
A9	0.59	0.57
A10	0.56	0.41

The corresponding three-dimensional representation of the desirability function obtained is shown in **Figure 4.10a**. This figure illustrates the effect of temperature and air rate on the desirability function. As observed, there is a maximum of predicted desirability (0.77) corresponding to a temperature value of 46 °C and an air rate of 4.9 m s^{-1}. The exact point can be better seen in **Figure 4.10b** which represents the corresponding contour plot. Among the tested conditions, the highest observed value of desirability function was 0.78, corresponding to A2 assay and 0.73 to the centred points (A3 and A6). Granato et al. (2010) obtained a value of desirability of 0.72 when studying the optimization of the sensory properties of dairy-free emulsions. Others authors such as Kargozari et al. (2010), optimizing physical properties of osmotically-dehydrated carrot cubes, obtained a desirability value of 0.92.

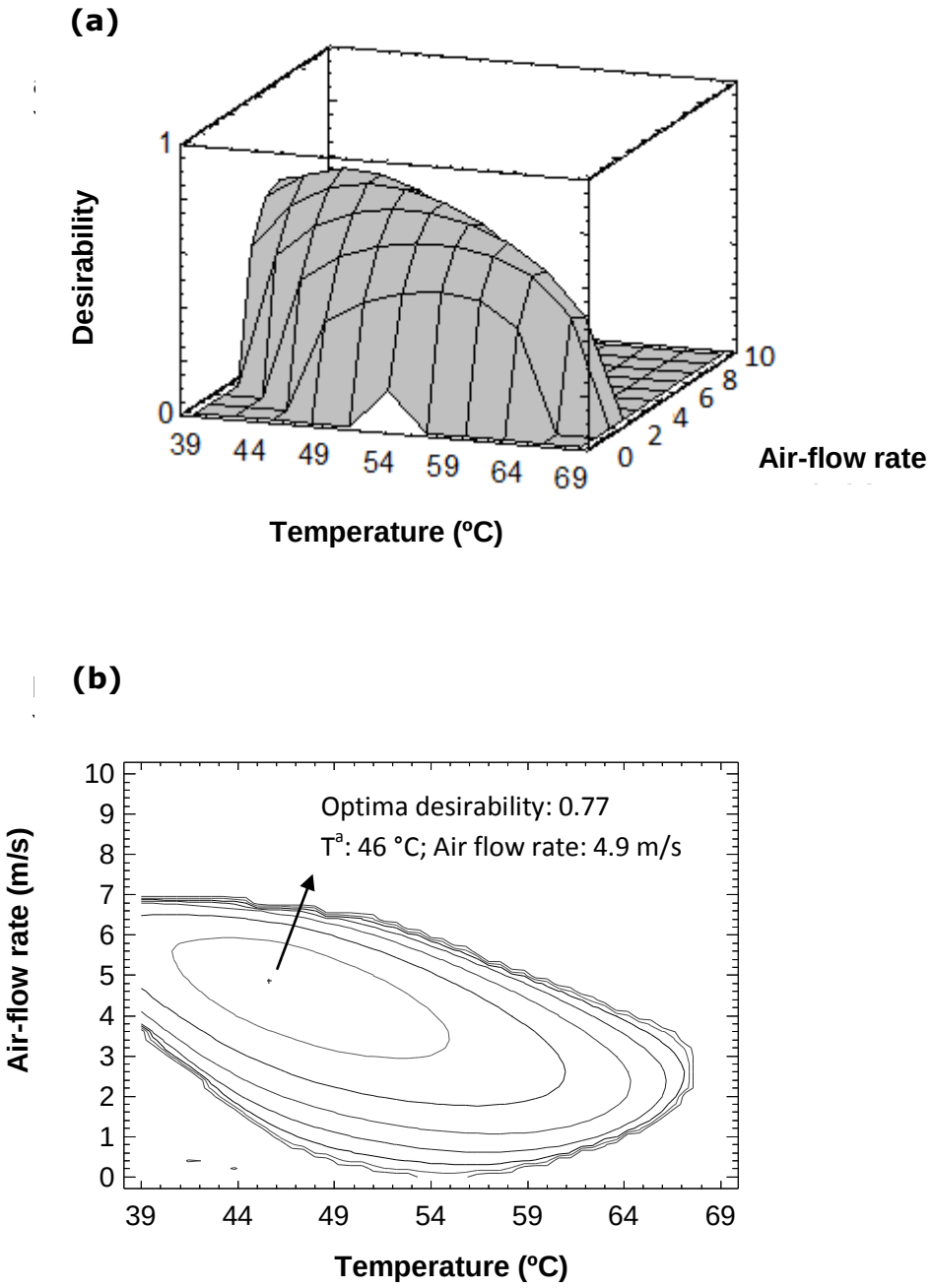

Figure 4.10 Estimated response surface (a) and the corresponding contour plot (b) for the desirability function.

In summary, **Table 4.15** shows the optimal values for the dependent variables obtained by RSM optimization of the process at 46 ºC and 4.9 m s^{-1}.

Table 4.15 Optima values of the different dependent variables to obtain the maximum desirability value corresponding to 46 °C and 4.9 m s^{-1}

Variables*	Values
S (kg H_2O kg^{-1} DM min^{-1})	0.052
W_1 (%)	35.1
2-FM-Lys+2-FM-Arg (mg kg^{-1} protein)	153
Vitamin C (mg kg^{-1} DM)	189
ß-carotene (mg kg^{-1} DM)	376

*S constant rate period of drying, W_1 weight loss at the first hour of processing, 2-FM-AA, 2-furoylmethyl-amino acids.

Conclusion

An experimental design is here proposed to optimize the operating conditions (temperature and air rate) with regard to selected processing (t*, S and W_1) and quality indicators (advance of MR and loss of vitamins). Furthermore, and in order to better determine the first drying period with constant rate, which can be described by a linear regression (R^2 = 0.99) of the drying curve, experimental quantification of shrinkage has also been carried out. In general, mild changes in the advance of MR and the loss of vitamin C and β-carotene were observed, even under the most severe conditions assayed, together with moisture loss values within the limits established to guarantee the microbiological stability of the product. RSM analysis of the operating conditions and studied indicators allowed, with high accuracy, the determination of optimal drying parameters (46 °C and 4.9 ms^{-1}). This study underlines the usefulness of optimizing the convective drying of carrots to obtain a long shelf-life product with premium quality (the lowest loss of nutritive value) in the shortest time and with the lowest energy requirement.

4.1.1.2.2. Impacto de las condiciones de procesado sobre la pérdida de vitamina C y la formación de 2-furoilmetil derivados durante la deshidratación convectiva de fresas

Impact of processing conditions on the kinetic of vitamin C degradation and 2-furoylmethyl amino acid formation in dried strawberries

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Journal of Agricultural and Food Chemistry (submitted)

Abstract

The effect of drying conditions on the kinetic of vitamin C degradation and of 2-furoylmethyl amino acid (2-FM-AA) formation during the convective drying of strawberries has been studied for the first time. Rehydration ability of these samples was also assessed. Vitamin C was better preserved (~90% retention) in samples processed at 40-50 ºC for up to 7 h. 2-FM-AA of Lys, Arg and GABA, identified for the first time in dried strawberries, increased with the drying temperature and time, particularly for samples processed at 70 ºC (up to 949.1 mg/100 g protein). Influence of temperature/time conditions resulted the most predominant factors over moisture content to explain the evolution of MR and vitamin C loss in these samples, following, respectively, a zero and first-order kinetics. As supported by its lower activation energy 2-FM-GABA (55.9 kJ/mol) and 2-FM-Lys + 2-FM-Arg (58.2 kJ/mol) were shown to be more sensitive than vitamin C (82.1 kJ/mol). The correlations between these indicators were also described by simple linear regressions.

Introduction

Strawberry (*Fragaria* x *ananassa*) is one of the most widely consumed fruits in the world because of its pleasant organoleptic characteristics and nutritive value, vitamin C standing out with a content of about 60 mg/100 g

fresh weight (Proteggente et al., 2002). Moreover, in a number of studies in the last decades, strawberry consumption has also been related to human health benefits due to their antioxidant, anticancer, anti-inflammatory and anti-neurodegenerative properties (Hannum, 2004; Seeram, 2008; Basu et al., 2010; Suh & Pezzuto, 2012). As in the case of other fruits and vegetables, fresh strawberry availability is limited by its seasonal harvesting and short shelf-life. Therefore, strawberry is subjected to different industrial processes (freezing, drying, etc) to obtain a number of products that can either directly be consumed, or used as ingredients in a wide variety of foodstuffs such as cookies, cereals, energy bars, dairy products, beverages, jams and jellies.

Among the different processing techniques, dehydration of fruits by convective drying is one of the most popular. The removal of moisture avoids the growth of microorganisms and inhibits the activity of enzymes that can deteriorate the product during storage. Furthermore, drying transforms the fruit into a processed product with different characteristics, making thus easier its transportation and storage at ambient temperature (Jayaraman & Das Gupta, 1995). However, the processing conditions (temperature, air rate, humidity and time) selected in the application of this preservation method might also give rise to physical (shrinkage and hardness, among others) and chemical changes that directly affect the quality of the dehydrated product (Lin et al., 1998; Asami et al., 2003).

Vitamin C is one of the most important chemical indicators when evaluating the drying processing of fruits and vegetables. Degradation of this vitamin depends on several factors including temperature, oxygen, metal ion catalysis, light, moisture content, etc (Rojas & Gerschenson, 2001; Fano Castro et al., 2008). Furthermore, the retention of this vitamin in dried products is also assumed as a general indicator of the preservation of other less labile nutrients (Shitanda & Wanjala, 2006). One way to avoid excessive losses of this vitamin during processing is by means of the study of its degradation kinetic. In this concern, some authors have reported first-order kinetics to describe this reaction in several processed foods (Lee & Labuza, 1975; Haralampu & Karel, 1983; Mishkin et al., 1984; McMinn & Magee, 1997; Dadali & Özbek, 2009). Regarding the dehydration of strawberry, most of the studies available in the literature address the drying kinetic (Tsami &

Katsiodi, 2000; Doymaz, 2008b) and the effect of processing conditions on the final loss of this vitamin (Asami et al., 2003; Böhm et al., 2006; Wojdylo et al., 2009). However, to the best of our knowledge, no studies have previously been reported on the kinetic of vitamin C degradation during strawberry drying.

On the other hand, the low water activity (a_w) conditions and the high temperatures and long times of processing make favourable the evolution of Maillard reaction (MR), a non-enzymatic browning reaction that takes place between reducing carbohydrates and free amino groups of amino acids, peptides and proteins, during dehydration of fruits. In this respect, 2-furoylmethyl amino acids (2-FM-AA), derivatives of Amadori compounds formed in the first stages of MR, have been previously described as sensitive indicators of the early evolution of MR in several dehydrated fruits and vegetables (Sanz et al., 2001; Cardelle-Cobas et al., 2005; Rufián-Henares et al., 2008; Soria et al., 2009b; 2010; Wellner et al., 2011; Gamboa-Santos et al., 2013a; 2013b). Evaluation of 2-FM-AA provides very valuable information, as it early detection can prevent advanced stages of the MR in which important losses of nutritive value, mainly associated to the participation of the essential amino acid lysine in MR, are produced (Corzo-Martínez et al., 2012). However, to the best of our knowledge, these compounds have not previously been determined in dried strawberries.

At the sight of the above exposed, the aim of the present study was to investigate the effect of drying conditions on the kinetic of vitamin C degradation and of 2-FM-AA formation during the convective drying of strawberries. In addition, other quality parameters such as rehydration ability were also assessed in these samples.

Materials and Methods

Strawberry samples

Fresh strawberries (*Fragaria* x *ananassa* Duch.) were purchased from a local market in Madrid (Spain). They were stored in the dark at 4 °C for a maximum period of 3 days until dehydration. Fresh samples were washed in tap water to remove external impurities and, previously to convective drying,

they were cut into 2.5 ± 0.5 mm thickness slices along their longitudinal axes. Additionally, strawberry samples processed in a laboratory-scale freeze-drier (FD-LAB) were used as control. The moisture content of raw and dried strawberries was determined at 102 °C until constant weight (AOAC, 1990a). Water activity measurement was carried out in a standardized conductivity hygrometer NOVASINA TH-500 (Air Systems for Air Treatment, Pfäffikon, Switzerland) at 25 ºC. The device was previously calibrated using the following salts: LiCl, $MgCl_2$, $Mg(NO_3)_2$, NaCl, $BaCl_2$ and $K_2Cr_2O_7$ according to the calibration procedure of the equipment manufacturer.

Drying processing

Drying assays were carried out using a computer controlled (Edibon Scada Control and Data Acquisition Software) air tray dryer (SBANC, Edibon Technical Teaching Units, Spain) which has already been described in detail by Gamboa-Santos et al. (2012b). Briefly, the system consists of a fan unit with air rate control, seven sensors for temperature control and a load cell with two metallic meshs for automatically monitoring of sample weight. For strawberry drying experiments, temperature of the sensor closest to the sample was set in the 40-70 °C range (**Table 4.16**) and the treatment times were 1, 3, 5 and 7 h. The air flow was parallel to the samples and the air rate was set between 2 and 8 m/s. The initial weight of the samples was 76.8 ± 3.2 g and it was automatically monitored by the load cell. All drying experiments were carried out in duplicate.

Table 4.16 Processing conditions for convective drying of strawberries

Assay	Temperature (ºC)[1]	Air flow-rate (m/s)
A-40	43.8 ± 2.3	8.0
A-50	48.2 ± 0.6	6.0
A-60	57.1 ± 1.1	4.0
A-70	72.2 ± 1.9	2.0

[1]Temperature of the wet bulb closest to the sample.

Determination of vitamin C

The total vitamin C content (ascorbic acid plus dehydroascorbic acid) of strawberry samples was determined following the method of Gamboa-Santos et al. (2013b). The reduction of dehydroascorbic acid to ascorbic acid was carried out with D,L-dithiothreitol as reducing reagent. Extracts were prepared by adding 12.5 mL of 0.4% oxalic acid to 0.25 g of freeze dried strawberries. Samples were homogenised for 1 min at 13,500 rpm using an Ultra-Turrax T-25 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany). After addition of 2.5 mL of a 5 mg/mL solution of D,L-dithiothreitol, strawberry extracts were kept at room temperature in the darkness for 30 min. Once the volume of the slurries was made up to 25 mL with Milli-Q water, they were centrifuged at 3,200*g* for 5 min. The supernatants were filtered through 0.45 µm syringe filters. Sample extracts were made in triplicate.

Vitamin C analyses were performed by Reversed Phase-High Performance Liquid Chromatography with Diode Array Detection (RP-HPLC-DAD) on an Agilent Technologies 1220 Infinity LC – 1260 DAD instrument (Boeblingen, Germany). Separation was carried out under isocratic conditions (flow rate 1 mL/min; 10 min) on an ACE 5 C_{18} column (ACE, UK) (250 mm length x 4.6 mm i.d. x 5 µm) at 25 °C, using 5 mM KH_2PO_4 (pH 3.0) as mobile phase. Injection volume was 20 µL and data were acquired and processed using the Agilent ChemStation software (Agilent Technologies, Germany).

Quantitation was performed by the external standard method, using a commercial standard of ascorbic acid (Sigma Chemical Co., St. Louis, US) in the range 0.3–50 mg/L. Determination coefficient from this calibration curve, which was linear over the range studied, was R^2 = 0.999. Vitamin C content was expressed as relative variation from raw control material.

Determination of 2-furoylmethyl amino acids

Strawberry hydrolysates were prepared in Pyrex screw-cap vials provided with polytetrafluoroethylene-faced septa by adding 4 mL of HCl 8 M to 0.25 g of each of the strawberries under analysis. Hydrolysis of samples under nitrogen atmosphere was complete after 23 h at 110 °C (Gamboa-

Santos et al., 2013a). Once the resulting hydrolysates were filtered (paper filter Whatman no. 40), 0.5 mL of each hydrolysate were purified by using a Sep-Pack C_{18} cartridge (Millipore, MA) previously activated with 5 mL of methanol and 10 mL of water. Finally, filtrates were eluted with 3 mL of 3 M HCl and 50 µL were injected into the chromatograph by means of a manual Rheodyne valve.

Ion-Pair Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) analysis of 2-FM-AA was done according to Resmini & Pellegrino (1991). Separation was carried out in a furosine-dedicated C_8 column (250 mm length x 4.6 mm i.d., Alltech, Lexington, KY) at 37 °C. The linear binary gradient of phase A (4 mL/L acetic acid) and phase B (3 g/L KCl in phase A solution) was as follows: 100% A between 0 and 12 min; 50% A from 20 to 22.5 min; 100% A for 24.5 to 30 min. The flow rate was 1.2 mL/min and detection was done at 280 nm using a variable wavelength detector (LCD Analytical SM 4000).

Quantitation was performed by the external standard method, using a commercial standard of furosine (Neosystem Laboratoire). Data were expressed as mg/100 g protein and all the analyses were performed in duplicate. Total nitrogen (TN) was determined by the Kjeldahl method (AOAC, 1990b), and the protein content of strawberries was calculated using 6.25 as conversion factor (TN x 6.25).

Rehydration properties

Strawberry slices were rehydrated in Milli-Q water (solid-to-liquid ratio 1:50) at room temperature for 2 hours. After removing the superficial water with tissue paper, the rehydrated strawberries were weighted. For each rehydration experiment (n = 3), the rehydration ratio (RR) was calculated as follows:

$$RR = \frac{m_r}{m_d} \quad (1)$$

where m_r and m_d represent the mass (g) of rehydrated and dehydrated strawberry, respectively.

Determination of soluble solids lost during rehydration (LL) was carried out as follows: 0.5 mL of soak water of each rehydration experiment was dried in a conventional oven at 102 °C for 24 h. The final solid residue was weighted to calculate the percentage of leached solids with respect to the initial weight of dried strawberry.

Kinetic modelling

In order to predict the changes in the content of 2-FM-AA and of vitamin C during drying of strawberries, the zero and first-order reaction models were respectively applied, assuming previous related studies (Montilla et al., 1996; McMinn & Magee, 1997). The respective equations for 2-FM-AA formation and vitamin C degradation are shown below,

$$\frac{dC_1}{dt} = k_1 \tag{2}$$

$$-\frac{dC_2}{dt} = k_2 C_2 \tag{3}$$

where C_1 is the concentration of 2-FM-AA and C_2 is the concentration of Vitamin C, at any time t. k_1 and k_2 are the reaction rate constants for 2-FM-AA formation and vitamin C degradation, respectively.

The temperature dependency of the reaction rate constants was determined by the Arrhenius-type equation (Devahastin & Niamnuy, 2010) (Eq. 4).

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \tag{4}$$

where k_0 is the pre-exponential Arrhenius factor, E_a is the activation energy (kJ/mol), R is the ideal gas constant (kJ mol/ºK), and T is the temperature (ºK).

Statistical analysis

Goodness of fittings was evaluated by means of the correlation coefficient *R* and the mean relative error (*MRE*) calculated from Eq. (5).

$$MRE = \frac{100}{N}\left[\sum_{i=1}^{N}\frac{|Y_{ei} - Y_{ci}|}{Y_{ei}}\right] \quad (5)$$

where Y_{ei} and Y_{ci} are the experimental and calculated variables (average moisture, *W*; vitamin C; 2-FM-GABA or 2-FM-Lys + 2-FM-Arg contents) and N is the number of experimental data.

To evaluate differences among samples data were subjected to one-way analysis of variance (Fisher's Least Significant Difference Test) by applying the Statgraphic 5.0 program (Statistical Graphics Corp., Rocville, MD). The significance of differences was defined as $p < 0.05$.

Results and discussion

Drying kinetic

Figure 4.11 shows the drying curves obtained during the processing of strawberries in the prototype of convective dehydration described in *Materials and Methods* under the conditions listed in **Table 4.16**. For each assay, this figure illustrates the evolution of the moisture loss up to 7 h of drying. As observed in **Figure 4.11**, A-70 and A-60 assays presented the highest slopes, whereas the mildest assays (A-50 and A-40) gave rise to a lightly slower drying rate. After 3 h of drying, strawberries showed DM contents > 80% in the case of A-70 and A-60 assays and > 75% in A-50 and A-40 experiments; these values were very close to that generally considered for microbiological stability of dried products (85%) (Belitz et al., 2009a). Moreover, strawberry samples presented values of a_w similar to 0.3 after this time of drying and hardly any change was detected in this parameter during its further processing. In general, it has been described that modifications as non-enzymatic browning are avoided at a_w below 0.3 (Belitz et al., 2009a;

Corzo-Martínez et al., 2012) and a_w values lower than 0.210 can also slow down the degradation of different bioactive compounds (Moraga et al., 2012).

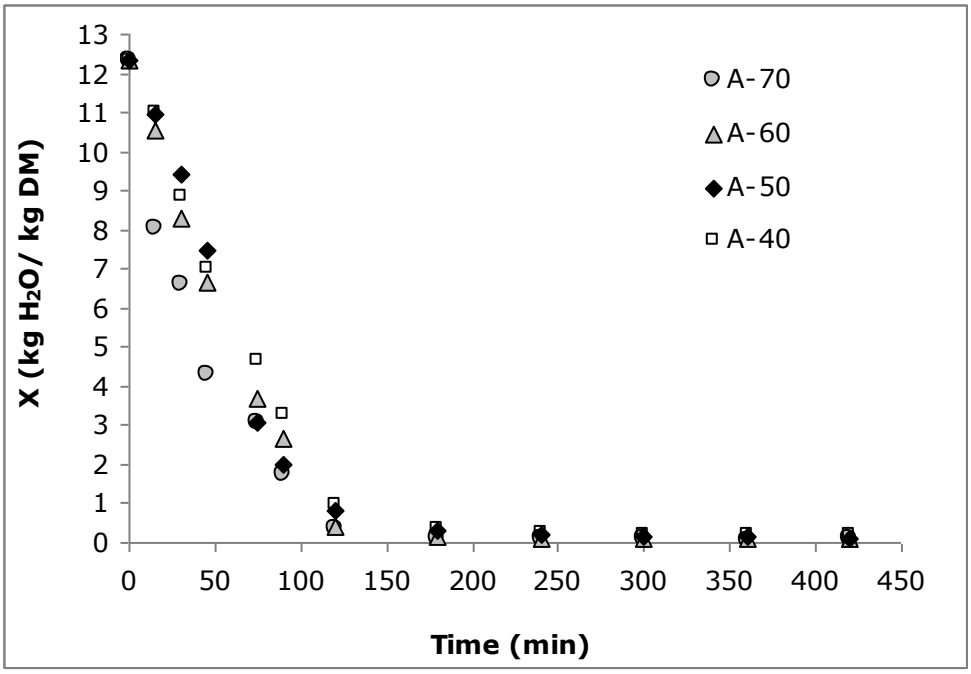

Figure 4.11 Drying curves up to 7 h for strawberry samples processed at different air-flow rates and temperatures (Table 4.16).

Degradation of vitamin C

In agreement with other investigations on the deterioration of nutritional quality during food processing, vitamin C was chosen in the present paper as a very sensitive and relatively easy-to-measure marker for determination of food quality (Ryley, 1989). The average vitamin C content determined in the raw strawberry samples analysed in this paper was 590.9 ± 7.4 mg/100 g DM. This value was close to those reported by other authors (635-683 mg/100 g DM, Böhm et al., 2006; 340-680 mg/100 g DM, Wojdylo et al., 2009). **Figure 4.12** shows the percentages of vitamin C retention (relative to the average raw control) calculated for strawberries processed under the different drying conditions assayed.

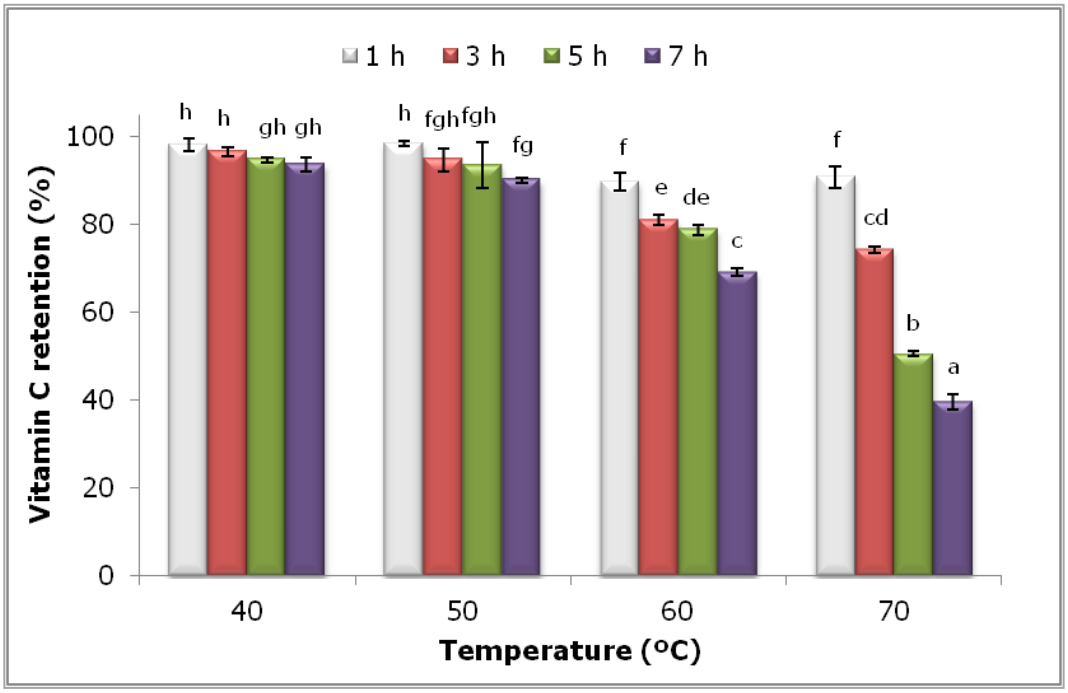

Figure 4.12 Vitamin C retention (%) in dried strawberry samples under analysis (mean of three replicates ± SD in bars). Samples with the same letter (a-h) within the same drying temperature showed no statistically significant differences for their mean values at the 95.0% confidence level.

Concerning the effect of moisture content on the degradation of vitamin C, the mechanism by which water controls the degradation reaction is very complex and it is dependent on the complexity of the plant tissue, the pre-processing history and, particularly, the specific moisture range (McMinn & Magee, 1997; Santos & Silva, 2008). Thus, in a study on the drying of tomato, Goula & Adamopoulos (2006) found that the reaction rate of ascorbic acid degradation increased with the reduction of moisture content from 95 to 65%. When moisture content reached 65-70%, the rate of this reaction reached a maximum value and at moisture contents below 65%, the rate decreased with moisture reduction. In our assays, when the time of drying was 3 h or higher, a very low moisture content (lower than 65%) was detected in all strawberry samples analysed, suggesting that, with the exception of the first hour of drying in which the moisture was high, hardly any effect of this parameter on the degradation of vitamin C can be expected. On the other hand, Santos & Silva (2008), in a review on retention of vitamin C in drying processes of fruits and vegetables, confirmed that at the beginning of the process, the effect of moisture content seems to be

predominant, while the temperature effect becomes major as the process proceeds. As observed in **Figure 4.12**, the retention of vitamin C was reduced with the time and temperature of drying; this trend was particularly evident at the end of the processes carried out at 60 and 70 °C with retention values of 69 and 40%, respectively. It is also remarkable the high retention of vitamin C (close to 90%) at the mildest temperatures (40 and 50 °C), irrespective of the time of processing. Böhm et al. (2006) observed an ascorbic acid retention of 31-42% with respect to its initial value for strawberries of different varieties (Camarosa, Darselect and Senga Sengana) subjected to a convective drying at 60 ºC, 5 m/s during 220 min. Wojdylo et al. (2009), in a comparative study on several procedures of drying, found a retention of ascorbic acid close to 30% in samples of Elsanta and Kent strawberry dried by convection at 70 °C for 8 h. Serious losses of ascorbic acid content (retention 13-16%) have also been reported after the convective drying of strawberries (Northwest Totem) for a total time of 88 h at temperatures of 49 °C and 77 °C (Asami et al., 2003). As compared to our data, the differences observed could be due to factors such as strawberry variety, maturity degree, geometry of samples, equipment characteristics and processing conditions, among others.

In order to evaluate the nutritional value of the samples processed in this study, and taking into account that even under the most severe conditions (70 °C, 7 h) an important concentration of vitamin C (233 mg/100 g DM; 40% retention) was preserved, calculation of the minimum amount of dried strawberry required to cover the recommended daily intake (RDI) of this vitamin was done. RDI of vitamin C has been reported to be 40 mg in Australia and United Kingdom (Australia and New Zealand Food Authority, 2001; Ministry of Agricultural, Fisheries and Food, 1995) and 60 mg in the United States (FDA, 1998). Therefore, and in absence of other alternative sources, the necessities of daily intake of vitamin C are fully covered with 21-32 g of dried strawberries (80% DM).

Taking into account the evolution of vitamin C retention during the drying process in the present paper (**Figure 4.12**) and, in agreement with other authors who have studied the kinetic of degradation of this vitamin in dehydrated model systems (Dennison & Kirk, 1978), dried potato (McMinn & Magee, 1997; Khraisheh et al., 2004), rosehip (Erenturk et al., 2005),

guavas (Sanjinez-Argandoña et al., 2005), tomato (Goula & Adamopoulos, 2006) and kiwi (Orikasa et al., 2008), data were fitted to a first-order kinetic model (**Figure 4.13**).

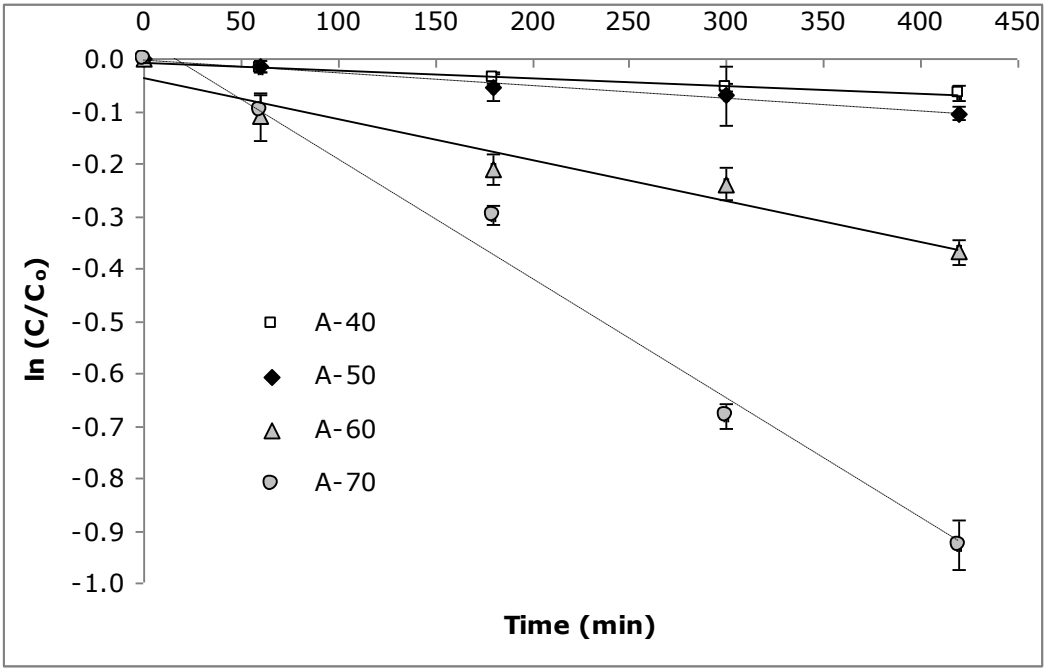

Figure 4.13 Kinetic of vitamin C degradation in strawberry samples dried under different experimental conditions (Table 4.16).

As observed, the experimental ln C/C_0 versus time representation exhibited linear correlations for each temperature, with slopes equivalent to the rate constant (*k*) and correlation coefficients (*R*) higher than 0.97 (**Table 4.17**), suggesting that the model was satisfactory in describing the degradation of vitamin C during convective drying of strawberries. The *k* values were very low in the case of processes carried out at 40 and 50°C, indicating that, under these conditions, vitamin C was not as sensitive as in the other tested temperatures (60 and 70°C). In a study on starchy food drying, Khraisheh et al. (2004) found *k* values in the range 0.0016-0.0018 min^{-1} for ascorbic acid degradation at temperatures 30-60 °C.

Table 4.17 Kinetic parameters determined for strawberries convectively dried at 40-70 ºC. Reaction rate constant (*k*), correlation coefficient (R) and mean relative error (MRE) for the fitting of data on vitamin C degradation and 2-FM-AA formation according to first and zero order reactions

Assay	$k_{vit\ C}$ (min^{-1})	R	MRE (%)	$K_{2\text{-FM-Lys+2-FM-Arg}}$ (mg/100 g protein · min^{-1})	R	MRE (%)	$K_{2\text{-FM-GABA}}$ (mg/100 g protein · min^{-1})	R	MRE (%)
A-40	-0.00015	0.986	7.97	0.1515	0.965	11.07	0.1561	0.999	2.61
A-50	-0.00024	0.992	6.48	0.3075	0.994	4.89	0.2825	0.983	11.76
A-60	-0.00079	0.972	11.29	0.3962	0.995	4.68	0.3428	0.992	6.49
A-70	-0.00228	0.993	7.08	1.2601	0.994	4.51	1.0698	0.998	2.41

With the purpose of gaining insight for the temperature dependence of vitamin C degradation during convective drying of strawberry, Arrhenius correlation was applied and the corresponding activation energy (E_a) calculated from the slope of the fitting. The E_a value (82.1 kJ/mol) here determined (R = 0.996) was comparable to data previously described by several authors. Lee & Labuza (1975) and Dennison & Kirk (1978) reported values of E_a in the wide range 7.5-125.6 kJ/mol for the thermal destruction of ascorbic acid in different dehydrated model systems. In kiwifruit, Orikasa et al. (2008) investigated the drying characteristics of kiwifruit during hot air drying at temperatures between 40 and 70 °C, and the E_a for the decomposition of ascorbic acid was estimated to be 38.6 kJ/mol. The difference between this result and the value of E_a obtained in the present work could be attributed to the different fruit considered, the processing system and geometry of samples, among other factors.

Formation of 2-furoylmethyl amino acids

Figure 4.14 depicts the RP-HPLC profile of 2-FM-AA present in the acid hydrolysate of the strawberry sample dehydrated at 60 ºC for 7 h at an air flow of 4 m/s. As described under *Materials and Methods*, identification of 2-FM-AA derivatives of γ-aminobutyric acid (peak 1) and of lysine plus arginine (peak 2) was done with different degrees of certainty. Tentative identification of 2-FM-GABA and of 2-FM-Arg was done by comparing the experimental retention times with data for standards previously analysed in our laboratory under identical experimental conditions (Soria et al., 2010) and considering data on free amino acid composition of strawberry (Keutgen et al., 2008; Blanch et al., 2012). Spiking of a tomato hydrolysate (Megías-Pérez et al., 2012), whose 2-FM-AA composition had previously been characterized (Sanz et al., 2000), was also done to support the tentative identification of 2-FM-GABA. Confirmation of the assignation of 2-FM-Lys was supported by data reported in the literature and by coinjection of a commercial standard. As far as we know, this is the first time that assignation of 2-FM-AA has been addressed in processed strawberries.

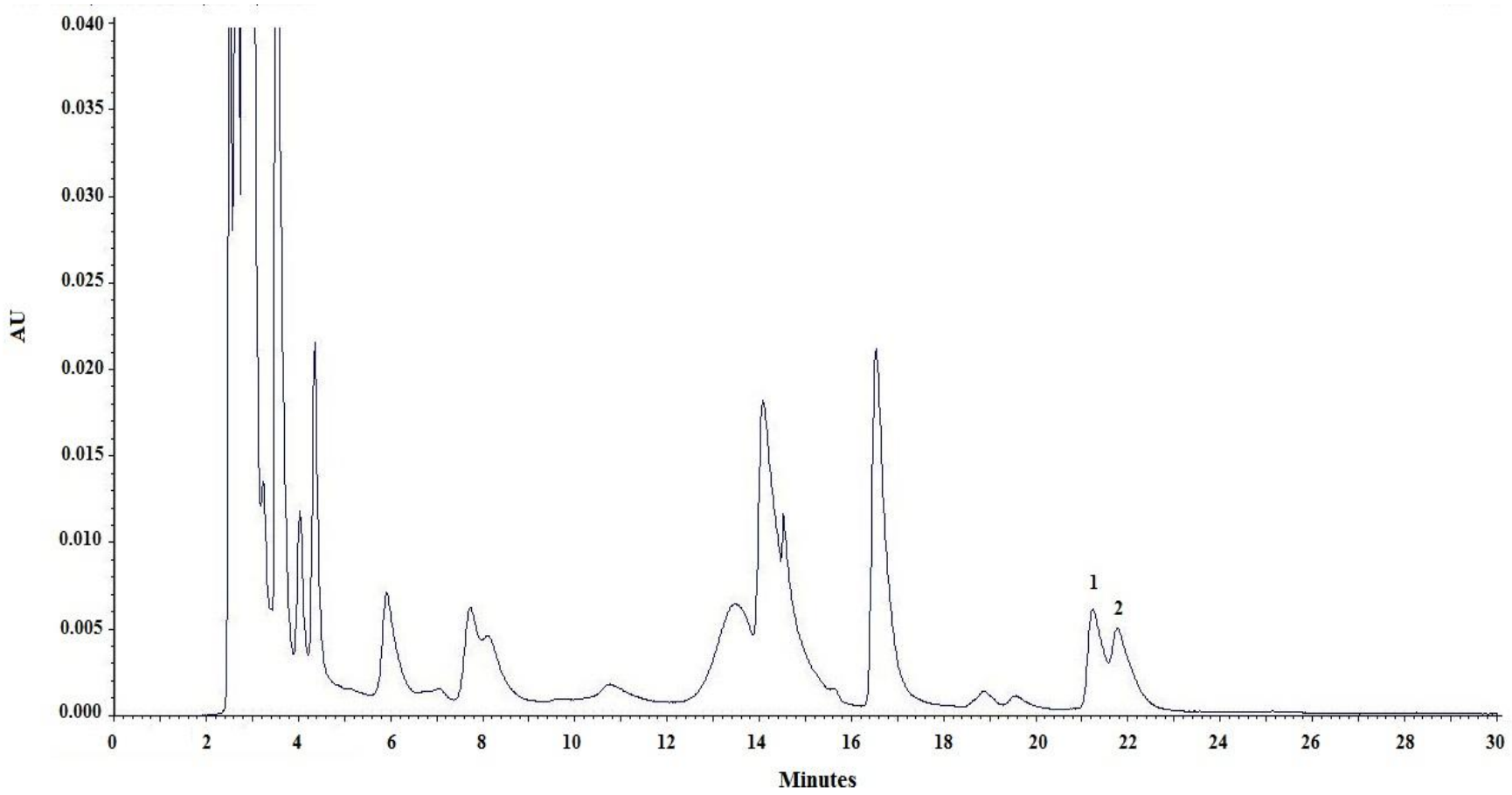

Figure 4.14 RP-HPLC-UV profile of the acid hydrolysate of strawberry sample dehydrated at 60 ºC for 7 h and 4 m/s. Peak 1, 2-FM-GABA, peak 2, 2-FM-Lys + 2-FM-Arg.

As observed in **Figure 4.15a,** the amount of 2-FM-Lys plus 2-FM-Arg determined in strawberries subjected to dehydration increased with the time and temperature and was found to be in the range 35.2-512.1 mg/100 g protein (2.7-38.8 mg/100 g product) for treatments at 40-70 ºC for 7 h. Sanz et al. (2001) reported values of these parameters in the range 7.7-93.4 mg/100 g product for dehydrated raisins, apricots, dates and figs; the different composition and processing of these last fruits could mainly justify the differences observed with respect to strawberry samples. Data for dried strawberries here analysed were also similar to those previously described by Gamboa-Santos et al. (2013a) for blanched carrots dried in the same prototype at 46°C and an air rate of 4.9 m/s for 7-9 h (104.3-681.5 mg/100 g protein). Considering foodstuffs derived from strawberry, Rada-Mendoza et al. (2002) reported a furosine content of 81.7 mg/100 g protein in strawberry jam. However, it is worth noting that the processing of this product is completely different and its a_w noticeably higher (0.919). The formation of 2-FM-GABA (**Figure 4.15b**) followed the same trend as that of the other 2-FM-derivatives previously mentioned, with values between 29.8 and 437.0 mg/100 g protein (2.3-33.1 mg/100 g product) detected in samples dried at 40 and 70 ºC up to7 h. Sanz et al. (2001) reported contents of this quality marker in the range 3.6-75.8 mg/100 g product for dehydrated raisins, apricots, dates and figs.

With respect to the effect of moisture content on 2-FM-AA formation, as above indicated for vitamin C degradation, for a given temperature, the main loss of moisture was produced before the three first hours of drying and scarce changes in 2-FM-AA content were observed after this time. However, a noticeable formation of 2-FM-AA was detected up to the end of the drying assays, especially at 70 ºC. Numerous studies have been conducted to address the complex moisture-dependent characteristics of the non-enzymatic browning reactions. Labuza et al. (1970) demonstrated that at low (due to limitations of reactants) and high (due to dilution effects) moisture contents the reaction rate decreases. Troller (1989) established "critical moisture contents" for browning to be produced in dehydrated food systems within the a_w range 0.65-0.75. In our assays, after three hours of drying the a_w was close to 0.3 and this value remained almost constant until the end of the process; therefore, it seems that the combination of temperature/time

conditions seems to exert a predominant effect over a_w in convective drying of strawberries.

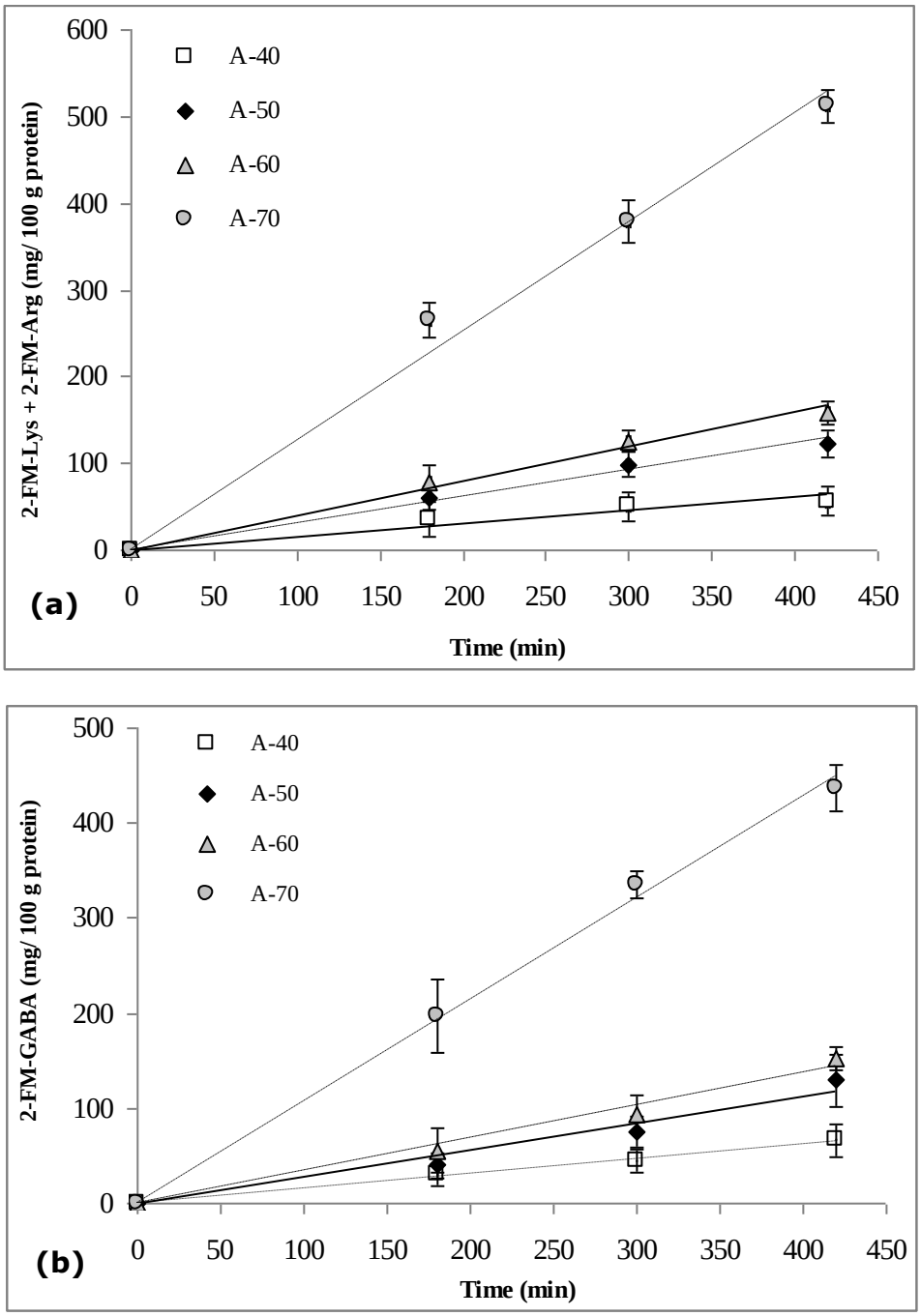

Figure 4.15 Evolution with time of the 2-FM-AA content of strawberry samples dried under different experimental conditions (Table 4.16): (a) 2-FM-Lys + 2-FM-Arg, (b) 2-FM-GABA.

Data on the formation of 2-FM-Lys plus 2-FM-Arg (**Figure 4.15a**) and of 2-FM-GABA (**Figure 4.15b**) during drying of strawberry samples were adjusted to zero-order reaction models and the rate constants obtained,

together with the corresponding determination coefficients and MRE values (**Table 4.17)**. In general, for all the temperatures assayed, a good fitting of the data was obtained with *R* higher than 0.97 and MRE values below 12%. As expected, *k* values for 2-FM-Lys + 2-FM-Arg and for 2-FM-GABA notably increased with temperature and, from the Arrhenius plot, the temperature-dependence of the formation of 2-FM-AA was corroborated. The E_a values calculated from the corresponding Arrhenius equations were 58.2 (R = 0.94) and 55.9 kJ/mol (R= 0.94) for 2-FM-Lys plus 2-FM-Arg and for 2-FM-GABA formation, respectively. To the best of our knowledge, no previous data have been reported on the kinetic of formation of 2-FM-AA in dried fruits. The only E_a data for furosine formation are those reported by other authors in heated milk (93-104 kJ/mol) (Montilla et al., 1996; De Rafael et al., 1997), tomato products (94 kJ/mol) (Hidalgo & Pompei, 2000), fresh filled pasta (111 kJ/mol) (Zardetto et al., 2003) and infant formula (113 kJ/mol) (Damjanovic Desic & Birlouez-Aragon, 2011). As in the case of dairy products, where lactose is less reactive to MR than glucose and fructose present in dried strawberries, the different composition of all these food stuffs could justify the higher E_a of furosine reported. With respect to vitamin C degradation, the E_a was higher (82.1 kJ/mol) than those of 2-FM-AA formation. In agreement with E_a values it could be deduced, that the latter are slightly more sensible parameters during drying of strawberries in the conditions here assayed.

As it is known, the correlation of diverse quality indicators has shown to be a good tool for the control of several food preservation processes. In this sense, no correlation has previously been evaluated between vitamin C degradation and 2-FM-AA formation or between the formation of different 2-FM-AA. Taking into account the parameters analysed in this paper, their correlation (**Tables 4.18** and **4.19**) can be adequately described by simple linear regressions. Regarding the fitting of experimental data summarized in **Table 4.18**, no clear trend associated with temperature could be established between vitamin C loss and 2-FM-AA formation. With respect to the correlation of 2-FM-AA (**Table 4.19**), a certain trend was observed, since at low temperatures 2-FM-GABA could be as sensitive as 2-FM-Lys + 2-FM-Arg and, at high temperatures, the formation of 2-FM-Lys + 2-FM-Arg could be favoured over that of 2-FM-GABA.

Table 4.18 Correlation of 2-FM-AA formation as a function of vitamin C degradation in strawberry samples under analysis. Correlation coefficient (*R*) and mean relative error (*MRE*) of the fitting

Assay	2-FM-Lys + 2-FM-Arg	R	MRE (%)	2-FM-GABA	R	MRE (%)
A-40	-1.52*VitC + 914.80	0.99	2.99	-1.65*VitC + 988.44	0.99	7.31
A-50	-2.17*VitC + 1278.50	0.98	8.24	-2.20*VitC + 1288.13	0.97	14.55
A-60	-0.89*VitC + 519.28	0.98	9.55	-0.82*VitC + 468.80	0.96	14.89
A-70	-1.37*VitC + 819.61	0.99	6.68	-1.20*VitC + 707.74	0.99	3.32

Table 4.19 Correlation of 2-furoyl-methyl amino acids (2-FM-GABA and 2-FM-Lys+2-FM-Arg) in strawberry samples under analysis. Correlation coefficient (*R*) and mean relative error (*MRE*) of the fitting

Assay	2-FM-GABA	R	RME (%)
A-40	1.06*(2-FM-Lys + 2-FM-Arg) - 2.60	0.97	10.25
A-50	0.99*(2-FM-Lys + 2-FM-Arg) - 8.41	0.97	11.35
A-60	0.91*(2-FM-Lys + 2-FM-Arg) - 7.22	0.98	7.74
A-70	0.87*(2-FM-Lys + 2-FM-Arg) - 7.67	0.99	3.28

Rehydration properties

The rehydration ability of strawberry samples subjected to convective drying was quantified on the basis of the rehydration ratio (*RR*) and the leaching losses (*LL*) (**Table 4.20**). As it can be seen in this table, hardly any change in *RR* and *LL* were found to be associated with the increase of drying time for any of assayed processing temperatures. The best rehydration properties were determined for A-40 and A-50 assays, with *RR* values (6.3-7.0) close to that of the freeze-dried sample processed in our laboratory (6.8 ± 0.6). Similarly, leaching losses of freeze-dried control samples matched closely those of samples A-40 and A-50. Megías-Pérez et al. (submitted), in a survey on several quality indicators in commercial dried fruits, reported *RR* values from 4.3 to 6.9 and *LL* values in the range 59.7-72.4 g/100 DM for freeze-dried strawberry samples and worse values of these properties for convective dried samples. El-Beltagy et al. (2007) reported lower *RR* data

(within the range 2.57-3.44) for strawberry samples of different geometries subjected to solar drying for up to 24 h.

Table 4.20 Rehydration ratio (*RR*) and leaching losses (*LL*) (average ± SD, *n* = 3) of strawberry samples under analysis

Assay	Drying time (h)	*RR*	*LL* (g /100 g DM)
A-40	3	6.3 ± 0.3^{d1}	65.9 ± 2.1^{c}
	5	6.7 ± 0.3^{de}	62.0 ± 2.7^{ab}
	7	6.6 ± 0.4^{de}	60.7 ± 2.9^{a}
A-50	3	6.4 ± 0.4^{de}	59.9 ± 3.6^{a}
	5	6.8 ± 0.3^{e}	62.4 ± 0.2^{abc}
	7	7.0 ± 0.3^{e}	59.0 ± 5.1^{a}
A-60	3	4.4 ± 0.2^{ab}	70.2 ± 2.8^{d}
	5	4.4 ± 0.2^{ab}	70.4 ± 4.5^{d}
	7	4.6 ± 0.6^{b}	65.5 ± 4.9^{bc}
A-70	3	5.6 ± 0.3^{c}	72.1 ± 3.4^{d}
	5	4.0 ± 0.3^{a}	70.5 ± 2.4^{d}
	7	4.2 ± 0.3^{ab}	72.4 ± 1.1^{d}

[1]Samples with the same superscript letter within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

As observed in **Table 4.20**, assays A-60 and A-70 gave rise to a decrease of the rehydration ability of dried strawberry samples (*RR* = 4.0-5.6; *LL* = 65.5-72.4 g/100 g DM). This fact was probably due to the severity of the drying processes carried out under these conditions. Agnieszka and Andrzej (2010) reported significant structural changes in convective dried strawberry samples (60 °C, 1.8 m/s air flow, 3 h) as compared to freeze-dried fruits. Thus, in a Scanning Electron Microscopy (SEM) analysis, these authors observed radical changes in the structure, with tearing of the cellular walls and part of the tissue as an homogeneous and compact substance, in strawberries processed under these conditions. In agreement with this, Jokic et al. (2009) reported a decrease of *RR* (from 6.9 to 5.9) with the increase of drying temperature (50-70 ºC) in dried apples not subjected to any pre-treatment. A similar effect was also described by Vega-Galvez et al. (2009) in dried peppers processed at 50 and 90 ºC, without blanching. As an explanation for this, the damage in cellular structure might result in modification of osmotic properties of the cell as well as in lower diffusion of water through the surface during rehydration (Kaymak-Ertekin, 2002). It was also reported by these authors that, in general, rehydration rate decreases as the dehydration rate increases. The cellular

structure damage would also explain the higher *LL* determined in samples here analysed at 60 and 70 ºC.

Conclusions

The kinetic study of vitamin C degradation (first-order) and of 2-FM-AA formation (zero-order) during the drying of strawberry samples, addressed for the first time in this paper, highlights that both parameters are important markers for the quality control of strawberries dried under different operating conditions. According to the E_a, 2-FM-AA seem to be more sensitive parameters than vitamin C during the drying of strawberry by convection. From a practical point of view, and considering the correlations among indicators here determined, it is possible to carry out for most of drying conditions assayed the determination of one of these quality markers and the estimation of the other from the obtained regressions for strawberries dried under identical conditions. Regarding the rehydration ability, considered in this paper as a complementary quality indicator to nutritional markers (vitamin C and 2-FM-AA), the processing temperature seemed to exert a higher influence than drying time (over these parameters). The data here presented afford useful information to the optimization of convective drying of strawberries with the aim to obtain a product with high nutritive quality and bioactivity.

4.2. Escaldado de zanahoria mediante ultrasonidos de potencia. Efecto en su posterior secado por convección

4.2.1. Prefacio

Como es sabido, el escaldado es un tratamiento previo ampliamente utilizado en numerosos procesos a los que se someten los vegetales, entre ellos la deshidratación, y tiene como principal finalidad inactivar enzimas que podrían ocasionar un deterioro del vegetal a lo largo de su período de vida útil. Sin embargo, los tratamientos convencionales que se emplean en la industria con este fin pueden ocasionar distintas modificaciones químicas y físicas dependiendo de las condiciones de tiempo y temperatura que se utilicen. En función del objetivo que se persiga y de la naturaleza del sustrato, las condiciones del proceso pueden ser más o menos enérgicas.

Con el fin de disponer de nuevos métodos de pre-tratamiento que permitan obtener vegetales con mejor calidad que la obtenida con los procedimientos tradicionales, durante la última década se han llevado a cabo investigaciones sobre la utilidad de los US en el escaldado y en la deshidratación osmótica de vegetales y frutas. En estos trabajos, se ha estudiado fundamentalmente la influencia de los US en el intercambio de materia durante el pre-tratamiento y la cinética de pérdida de humedad en un posterior proceso de secado convectivo. Además, también se ha investigado la aplicación de los US con temperatura (termosonicación) como alternativa a un escaldado convencional, determinándose su efecto sobre la inactivación microbiana. En el caso de zanahoria, se han realizado estudios sobre la retención de carotenoides y sobre las propiedades mecánicas del sustrato, cuando se emplean los US como pre-tratamiento. Hasta la realización de la presente Memoria, existían escasos trabajos sobre el impacto de los US en la inactivación de enzimas durante el pre-tratamiento de vegetales. Por ello, en la primera etapa de esta sección, se planteó un estudio en zanahoria (Apartado 4.2.1.1.1., *Effects of conventional and US blanching on enzyme inactivation and carbohydrate content of carrots*) para conocer el efecto de los US sobre enzimas que pueden ser indicadores del escaldado, como la POD o influir en la calidad del producto deshidratado, como la PME. Además, se determinaron las pérdidas de sólidos totales por

lixiviado y de la fracción de carbohidratos. Para llevar a cabo este estudio, se realizaron tratamientos con US en baño (40-60 °C) y con sonda (35-70 °C) a diferentes tiempos (10-60 min), además de tratamientos convencionales con vapor o con agua a distintas condiciones de temperatura y tiempo (HTST y LTLT). Respecto a la inactivación enzimática, se observó una mayor efectividad de los tratamientos llevados a cabo con sonda que los efectuados en baño, especialmente cuando se utilizaban los US con generación de calor, alcanzándose temperaturas de hasta 70 °C. En el caso de los tratamientos convencionales, la inactivación fue total bajo condiciones HTST. Lo más destacable de este estudio fue que se alcanzaron valores similares de inactivación enzimática y de pérdidas por lixiviado tras los tratamientos con US llevados a cabo con sonda durante 10 min y a temperaturas de hasta 60 °C, y tras los realizados convencionalmente bajo condiciones LTLT (60 °C, 40 min), indicando la utilidad de los US aplicados al pre-tratamiento en condiciones suaves.

En base a los resultados anteriores, se eligieron las condiciones de escaldado de zanahoria, tanto por US como convencionales, que permitían alcanzar la mayor inactivación de la POD, junto con unas pérdidas por lixiviado relativamente bajas. En dichas condiciones, se estudió el efecto de dichos pre-tratamientos sobre la cinética de la pérdida de humedad y sobre diferentes parámetros de calidad, químicos, físicos y sensoriales (Apartado 4.2.1.2.1., *Quality parameters in convective dehydrated carrots blanched by US and conventional treatment* y Apartado 4.2.1.2.2., *Vitamin C content and sensorial properties of dehydrated carrots blanched conventionally or by US*) durante la deshidratación de zanahorias bajo las condiciones optimizadas en el prototipo de secado por convección (Apartado 4.1.1.2.1., *Optimisation of convective drying of carrots using selected processing and quality indicators*).

En todos los casos se hallaron valores finales de humedad en las zanahorias deshidratadas dentro de los intervalos considerados microbiológicamente seguros. Además, no se observó actividad enzimática residual de la POD por lo que, de acuerdo a estos parámetros, los productos finales podrían ser estables a lo largo del período de vida útil. En relación a la cinética de pérdida de humedad, las muestras pre-tratadas con US y posteriormente secadas, presentaron velocidades de deshidratación superiores a las de zanahorias secadas tras escaldados convencionales con

vapor y ebullición, pero inferiores a los hallados en muestras escaldadas a 95 °C, 5 min y 60 °C, 40 min.

De los diferentes parámetros de calidad estudiados se prestó especial atención a la formación de 2-FM-AA, dada su utilidad como indicador del proceso de secado convectivo como se indicó anteriormente. No se detectó avance de la RM en las muestras pre-tratadas pero sí en las secadas. Los valores de 2-FM-AA fueron en todos los casos inferiores a los mostrados previamente en la literatura para vegetales deshidratados por convección. Se observó que las condiciones de escaldado a las que se somete el producto inicial afectan de forma significativa a la formación de los compuestos de Amadori durante la etapa posterior de secado. Las muestras pre-tratadas por US presentaron valores intermedios de 2-FM-AA. Los contenidos notablemente más elevados se obtuvieron en las zanahorias pre-tratadas a 95 °C, 5 min debido, probablemente, a alteraciones en la estructura de la proteína durante la fase previa de escaldado, tal y como indicó el análisis electroforético de la fracción proteica.

Otro de los parámetros químicos que se estudió fue la retención de la vitamina C. De los pre-tratamientos ensayados, los que evitaron una mayor pérdida de dicha vitamina fueron los llevados a cabo bajo ebullición o con vapor. Por lo que se refiere al efecto de los US, las muestras presentaron prácticamente una total pérdida de vitamina C, al igual que el tratamiento convencional LTLT. A pesar de las condiciones suaves de secado (46 ºC; 4,9 m/s), en las muestras de zanahoria escaldada que partían de retenciones altas, se observó una gran pérdida de vitamina C tras el secado. Esto fue debido, probablemente, a los elevados tiempos de procesamiento requeridos para alcanzar, en el producto final, una humedad que mantenga su estabilidad durante la conservación.

En relación a los parámetros físicos, las muestras deshidratadas previamente tratadas por US, presentaron valores de capacidad de rehidratación superiores a muestras liofilizadas y escaldadas con vapor y ebullición. Este hecho pudo deberse a modificaciones en el tejido de la zanahoria debido a la formación de microcanales a consecuencia del tratamiento con US, tal y como apuntaron los análisis microestructurales.

Por último, las muestras de zanahoria secadas, previamente escaldadas, fueron evaluadas por un panel de catadores semientrenados. La calidad

sensorial de las muestras pre-tratadas con US fue aceptable y similar a la de las zanahorias previamente escaldadas por procedimientos convencionales. Otra forma de evaluar la calidad sensorial es a través de sistemas de "pseudonariz electrónica" como es el ChemSensor. Mediante este sistema y técnicas quimiométricas es posible clasificar las muestras en función de su perfil de volátiles. Con este fin se procedió a estudiar las huellas de las fracciones másicas obtenidas tras un análisis por GC-MS de las muestras de zanahoria procesadas. El resultado más relevante fue que muestras deshidratadas con similar composición y/o similar procesado, indistinguibles para los panelistas, fueron perfectamente diferenciadas tras su análisis con el ChemSensor, indicando la utilidad de este sistema como herramienta para clasificar muestras de zanahoria procesada.

4.2.1.1 Efecto del escaldado convencional y por ultrasonidos sobre la inactivación enzimática y el contenido en carbohidratos de zanahorias

Effects of conventional and US blanching on enzyme inactivation and carbohydrate content of carrots

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European Food Research and Technology (2012), 234, 1071-1079.

Abstract

There is a growing interest in the use of US (US) as an alternative to conventional processes. Although US have previously been applied as a pre-treatment of fruits and vegetables, no investigation has been done on the usefulness of US for carrot blanching, paying special attention to its effect on enzyme inactivation and leaching losses. In the present paper, the influence of US (in bath and with probe) on peroxidase (POD) and pectinmethylesterase (PME) inactivation and on the loss of total soluble solids and carbohydrates by leaching has been evaluated. Results of this preliminary study have also been compared with those obtained after conventional (hot water and steam) blanching of carrots. The highest enzyme inactivation was obtained with the conventional treatments performed at high temperatures and with the US-probe treatments with heat generation. Carrots blanched by US-probe for 10 min at a temperature up to 60 ºC, showed similar characteristics than those conventionally treated at 60 ºC for 40 min. Although the efficiency of US was limited for total inactivation of POD and PME, this treatment resulted to be advantageous in terms of time for blanching at mild temperatures. US-probe treatments could also be considered as an advantageous alternative to low temperature-long time (LTLT) conventional treatments for those applications in which partial inactivation of PME is required for better preservation of carrot structure.

Introduction

Carrot (*Daucus carota* L.) is considered one of the most important vegetables due to its pleasant flavour, nutritive value and great health benefits related to its antioxidant, anticancer, antianemic, healing and sedative properties (Speizer et al., 1999; Shivhare et al., 2009). Carrot is constituted by, approximately, 90% of water and 5% of carbohydrates; vitamins and minerals, among other constituents, are also present at lower concentrations (Souci et al., 2009). Although carrots are widely consumed as fresh vegetables, due to their perishable nature, they are also subjected to different processes such as freezing, canning or dehydration to extend their shelf life for distribution and storage. Prior to these processes, carrots are usually blanched in hot water or steam for air removal, stabilization of colour, hydrolysis and solubilisation of protopectin and inactivation of microorganisms and enzymes (Bourne, 1976; Bahceci et al., 2005; Barret & Theerakulkait, 1995).

Enzymes such as peroxidase (EC 1.11.1.7, POD) and pectinmethylesterase (EC 3.1.1.11, PME) are of considerable importance since they can be involved in different degenerative modifications of vegetables (Fellows, 1994). Particularly, POD catalyses a great number of oxidation-reduction reactions and it is considered among the most heat-stable enzymes in plants. POD is widely used as an index of blanching since if this enzyme is inactivated, it is quite unlikely that other enzymes are active. Therefore, it has been accepted as a general rule in the food industry that if there is no activity of peroxidase, no activity of other heat-resistant enzymes such as catalase, should be detected. However, complete inactivation of peroxidase has been shown not to be necessary for quality preservation in frozen vegetables (Baardseth & Slinde, 1981). In relation to PME, this enzyme has an important role in textural changes of unblanched vegetables since it catalyses the de-esterification of pectin to pectic acid which facilitates the link of calcium and magnesium, increasing the firmness of the cellular wall (Alonso et al., 1995). In some cases, a certain residual PME is preferred since, after drying, the texture of rehydrated product can be improved (Lewicki, 2006; Lemmens et al., 2009); this is possible by blanching at low temperature and long-time (LTLT). Despite the beneficial effects of blanching

depend on the degree of thermal treatment applied, the quality and bioactivity of the final product can be negatively affected due to the destruction of nutrients relatively unstable to heat, the loss of water-soluble components by leaching and the changes in texture with this sample pre-treatment (Mizrahi, 1996; Wennberg et al., 2006).

On the other hand, as a result of the increased consumer's awareness of the relationship between diet and health, the food industry is greatly interested in the search for mild processing technologies which give rise to final products with improved characteristics as compared to those obtained by conventional thermal treatments, being high-intensity US (US) one of the emerging processes whose applications in the food industry have been recently reviewed (Soria & Villamiel, 2010). In this respect, there are some studies on the use of US as a pre-treatment before conventional drying and as a medium to assist osmotic dehydration of vegetable and fruits (Fernandes & Rodrigues, 2007; Jambrak et al., 2007a; Azoubel et al., 2010; Fernandes et al., 2011; Rawson et al., 2011). Most of these works have been carried out in ultrasonic baths at mild temperatures or have been mainly focused on the kinetic of moisture loss during drying; US showing a noticeable reduction in the overall drying time together with a variable loss of total sugars. In the case of carrots, hardly any research has been carried out on the potential of US as an alternative to conventional blanching with hot water or steam. Rawson et al. (Rawson et al., 2011) reported higher retention of carotenoids in hot air and freeze dried carrots previously subjected to US than in samples blanched with hot water at 80°C for 3 min. However, to the best of our knowledge, no previous work has been done on the effect of US on important enzymes related to carrot blanching. Therefore, this paper has been devoted: (i) to study the influence of US pre-treatments, with probe and in bath, on the inactivation of POD and PME, and (ii) to determine the changes in total soluble solids and major and minor carbohydrates of US-processed carrots. US pre-treatment results have been compared with those obtained in conventional heat blanching processes (steam and hot water 60-95 °C).

Materials and methods

Sample preparation

A big batch of fresh carrots (*Daucus carota* L. var. Nantesa) was purchased from a local market in Madrid (Spain) and was stored at 4 °C for less than a week until processing. Carrots were properly washed in tap water to remove external impurities. Then, samples were cut in slices of 24 mm in diameter and 4 mm thickness and as minced carrots (1-2 mm).

Processing

Table 4.21 summarizes all blanching processes (conventional and by US) carried out.

Table 4.21 Processing conditions used during the blanching of carrot samples by conventional and US (in bath and with probe) treatments

Blanching	Samples	Temperature (ºC)	Time (min)	US density (Wcm^{-3})[1]
Conventional	CS-2	Steam	2	-
	CB-1	98	1	-
	C95-5	95	5	-
	C60-40	60	40	-
US (in bath)	USB 40-30	40	30	0.04
	USB 40-60	60	60	
	USB 60-30		30	
	USB 60-60		60	
US (with probe)	USP 35-15	≤35	15	0.26
	USP 35-60		60	
	USP 60-10	≤60	10	
	USP 70-15	≤70	15	

[1]Determined according to Jambrak et al. (2007b)

Ultrasound treatments

For US treatments, samples of 40 g were added to the 250-mL Erlenmeyer flasks filled with 200 mL of distilled water. Two sets of experiments were carried out: (i) in bath and (ii) with an ultrasonic probe.

(i) Erlenmeyers containing the carrot samples were placed in a temperature-controlled US bath (30-70 ± 1 ºC) (SONICA SWEEP SYSTEM EP 2200, SOLTEC, Italy), operating at 45 kHz, and carrot samples were US-

treated at 40 and 60 °C for 30 and 60 min (USB 40-30; USB 40-60; USB 60-30; USB 60-60). The soak water was preheated at the selected temperature.

(ii) In the case of the assays with probe, Erlenmeyers with carrot samples were sonicated in an ultrasonic system (450 Digital Sonifier, Branson Ultrasonics Coorporation, Danbury, CT, USA). This sonicator is equipped with a temperature sensor (error ± 0.1 ºC) and a tip of 13 mm diameter directly attached to a disruptor horn (20 kHz, 400 W full power) and immersed 2 cm in depth with respect to the liquid surface (**Figure 4.16**). Experiments were carried out at low temperature (≤ 35 °C) for 15 and 60 min (USP 35-15, USP 35-60) by immersing the samples in an ice-water bath. Additional assays were done with generation of heat: temperatures up to 60 and 70 °C being achieved after 10 min (USP 60-10) and 15 min (USP 70-15) of sonication, respectively. In this case, the ice-water bath was removed.

The US density, calculated according to Jambrak et al. (2007b), was 0.04 and 0.26 Wcm^{-3}, respectively, for bath and probe experiments.

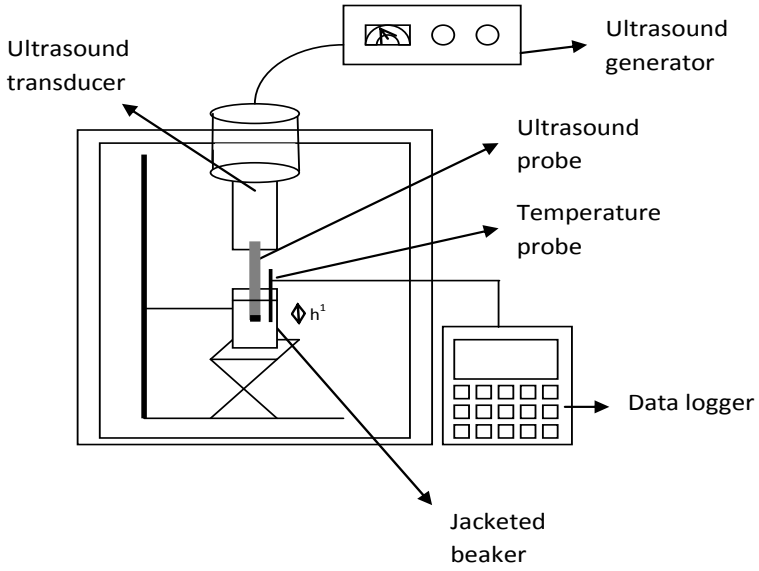

Figure 4.16 Experimental set-up for US treatments with probe.
[1]Depth of the probe in the sample (2 cm).

Conventional blanching treatments

Using the same carrot-distilled water ratio as above mentioned, carrot samples were subjected to blanching with boiling water for 1 min (CB-1),

with water at 95 °C for 5 min (C95-5) and at 60 °C for 40 min (C60-40) using a magnetic stirrer (200 rpm) with temperature control (IKA RCT Basic Labortechnik, Staufen, Germany). For CS-2 treatments (steam blanching), an autoclave (CERTOCLAV CV-EL GS, Austria) was used.

All assays (US and conventional) were performed in duplicate. After treatments, samples were cooled in an ice-water bath and conveniently drained and dried with absorbent paper to remove the excess of distilled water.

Sample characterization

The dry matter (DM) content of carrots was gravimetrically determined by drying the samples in a conventional oven at 102 ºC until constant weight (AOAC method, 1990a). The same method was used to determine the leaching loss during blanching. The percentage of leached solids was referred with respect to the initial weight of raw carrot (%).

The pH of blanching water was determined using a pH meter (Mettler-Toledo GMBH, Schwenzenbach, Switzerland).

Enzymatic determinations

Determination of peroxidase (POD) activity

The POD activity was determined as described by Shivhare et al. (2009) with slight modifications. Blanched carrots (2 g) were crushed in a domestic chopper (BRAUN, Germany) and, after addition of 5 mL of phosphate buffer solution (pH 6.5; 0.1 M), samples were homogenized for 30 s at 18000 rpm and 4 ºC using an Ultra-Turrax T-25 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany). The slurries were subsequently filtered through a medium-grade paper filter (Whatman no. 40) and the filtrates were centrifuged at 5,000*g* (Eppendorf, F-45-12-11, Hamburg, Germany) for 20 min. The POD substrate solution was daily prepared by mixing phosphate buffer solution (pH 6.5; 0.1 M), guaiacol (0.1% v/v) and hydrogen peroxide (0.1% v/v). The supernatants (60 µL) were added to 870 µL of enzymatic substrate solution. Residual POD activity was measured at 470 nm and 25 ºC in a spectrophotometer (Power Wave XS Microplate, BIO-TEK) using the KC

Junior Data Reduction software. The enzyme activity was determined from the slopes of linear progress curves generated on the recorder, and the slopes of raw samples were considered as indicatives of 100% of residual activity. The lower the value of the slopes calculated for blanched samples, the higher inactivation of POD in these samples. All determinations were carried out in duplicate.

Determination of pectinmethylesterase (PME) activity

The PME activity was determined in blanched carrots as described by Lemmens et al. (2009). Tris(hydroxymethyl-aminomethane) hydrochloride buffer (0.2 M; pH 8) containing 1 M NaCl was added to carrots (ratio buffer:carrots, 1.3-1). The samples were stirred for 2 h at 750 rpm and 22 ºC using a Thermomixer (Eppendorf, Germany). The supernatants were recovered after filtration (Whatman no. 40) and then used to measure the residual PME activity by a titrimetric method (pH 7 and 22 ºC). The enzymatic substrate (0.35% apple pectin solution, containing 0.125 M NaCl) was demethoxylated by the residual enzyme, and the released carboxyl groups were titrated with 0.01 M NaOH. The residual PME activity was expressed as percentage respect to the raw sample, which was considered with 100% activity. All extracts were made and titrated in duplicate.

Carbohydrate determination by GC

Carrot samples were freeze-dried and grinded to powders with a laboratory mill (Janke and Kunkel IKA A-10, Labortechnik, Staufen, Germany) and soluble sugars were extracted according to the method reported by Soria et al. (2010) with slight modifications. Grinded carrots (30 mg) were weighted in a polyethylene tube and extracted with 2 mL of Milli-Q water under stirring at room temperature for 20 min. Then, 8 mL of absolute ethanol were added followed by 0.2 mL of an ethanolic solution 10 mg mL^{-1} of phenyl-β-D-glucoside (Sigma Chemical Co., St. Louis, MO, USA) used as internal standard. After stirring for 10 min, samples were centrifuged at 10 °C and 9,600*g* for 10 min and the supernatant was collected. The precipitate was subjected to a second extraction with 10 mL of 80% ethanol under the same conditions to obtain recovery values close to 100%. Finally, 2

mL of supernatant was evaporated under vacuum at 40 °C. The extracts were prepared in duplicate.

The analysis was performed by GC as described by Soria et al. (2010) with a gas chromatograph (Agilent Technologies 7890A) equipped with a flame ionization detector (FID) and using nitrogen as carrier gas at a flow rate of 1 mL min^{-1}. The trimethylsilyl oxime (TMSO) derivatives, prepared as described by Montilla et al. (2009), were separated using a HP-5MS capillary column (5% phenyl methylsilicone, 30 m x 0.25 mm i.d. x 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). The oven temperature was held at 200 ºC for 11 min, then increased to 270 ºC at a heating rate of 15 °C min^{-1} and to 300 ºC at 3 ºC min^{-1} and finally raised to 315 ºC at 15 ºC min^{-1}, remaining at this temperature for 3 min. Injector and detector temperatures were 280 ºC and 315 °C, respectively. Injection was carried out in split mode (1:40).

Data acquisition and integration was done using Agilent ChemStation Rev. B.03.01 software (Wilmington, DE, USA). Identification of TMSO derivatives of carbohydrates was carried out by comparing the experimental retention indices with those of standards.

Quantitative data (mg g^{-1} DM) were calculated from FID peak areas. Standard solutions of fructose, glucose, sucrose, *scyllo*- and *myo*-inositol (all of them from Sigma Chemical Co.) over the expected concentration range in carrot extracts were prepared to calculate the response factor relative to the internal standard.

Soluble sugar content of blanching water (1 mL) was analysed using the same method, after addition of an ethanolic solution 0.5 mg mL^{-1} of phenyl-β-D-glucoside (0.4 mL) as internal standard. Samples were prepared in duplicate.

Statistical analyses

Data were subjected to one-way analysis of variance (Fisher's least significant difference (LSD) procedure) by applying the Statgraphic 4.0 software (Statistical Graphics Corp., Rockville, MD, USA) for Windows. The significance of differences was defined as $P < 0.05$.

Results and discussion

Effects of ultrasound and conventional blanching on enzyme inactivation

Table 4.22 lists the results corresponding to the enzymatic (POD and PME) activity of carrot samples subjected to the different blanching treatments under study. Considering POD activity, high temperature short time conventional blanching treatments (CB-1 and C95-5) gave rise to the total inactivation of this enzyme, in agreement with Kidmose and Martens (1999) and with Shivhare et al. (2009) that inactivated POD after 7 and 4 min at 80 and 90 °C, respectively. These authors also indicated that inactivation time of catalase and POD during steam blanching was consistently higher than in hot water. Similarly, in the present paper, some residual POD activity was detected in steam blanched carrots.

A certain effect of sample geometry was detected in samples subjected to conventional blanching treatments (CS-2, C60-40), with the highest inactivation of POD in minced as compared to sliced carrots (**Table 4.22**). The highest residual activity (40.9%) was observed for sliced carrots blanched at 60 °C for 40 min. Lemmens et al. (2009) reported residual POD activities of 70% after blanching treatments carried out under the same conditions, but with samples of 10 mm thickness.

In general, in the US blanching study, the reduction of POD activity was more evident for assays carried out with probe as compared to those with US bath, probably due to the higher acoustic density in the former experiments (0.26 Wcm^{-3} *vs.* 0.04 Wcm^{-3}). No inactivation of POD was detected in carrot samples US treated in bath at 40 °C (USB 40-30 and USB 40-60), while a significant inactivation of POD was observed at 60 °C, being this effect particularly noticeable after 60 min treatment of carrot slices. This could be due to the fact that, in minced carrots, the formation of sample aggregates, confirmed by visual inspection, might avoid the transfer of thermal and acoustic energy and, therefore, give rise to less cavitation phenomenon.

Table 4.22 POD and PME residual activity (%) in minced and sliced carrot samples after the different conventional and US blanching treatments. Mean of two replicates ± standard deviation.

	POD (%)		***PME (%)***	
Samples	***Minced***	***Sliced***	***Minced***	***Sliced***
Raw	100.0 ± 0.0^{d1}	100.0 ± 0.0^{d}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
CS-2	6.8 ± 1.6^{a}	15.4 ± 0.7^{a}	0.1 ± 0.1^{b}	0.2 ± 0.1^{b}
CB-1	1.0 ± 0.0^{b}	1.0 ± 0.0^{b}	0.1 ± 0.1^{b}	0.1 ± 0.1^{b}
C95-5	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
C60-40	12.4 ± 3.2^{c}	40.9 ± 6.4^{c}	62.9 ± 0.7^{cd}	56.8 ± 5.3^{c}
USB 40-30	100.0 ± 0.1^{d}	100.0 ± 0.2^{d}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
USB 40-60	100.0 ± 0.3^{d}	100.0 ± 0.1^{d}	79.9 ± 0.8^{g}	73.9 ± 8.0^{f}
USB 60-30	63.4 ± 6.9^{e}	25.5 ± 2.3^{e}	69.4 ± 4.8^{f}	52.4 ± 4.2^{e}
USB 60-60	63.4 ± 4.0^{e}	11.9 ± 0.5^{a}	68.4 ± 3.3^{df}	67.3 ± 1.6^{d}
USP 35-15	78.5 ± 5.7^{f}	71.4 ± 2.3^{f}	62.7 ± 1.2^{c}	61.8 ± 4.2^{c}
USP 35-60	58.3 ± 3.0^{g}	60.3 ± 8.2^{g}	49.0 ± 3.0^{e}	54.6 ± 4.3^{e}
USP 60-10	10.4 ± 0.1^{ac}	41.7 ± 8.4^{c}	69.1 ± 5.9^{f}	56.7 ± 8.0^{c}
USP 70-15	6.7 ± 1.4^{a}	17.4 ± 2.6^{a}	78.4 ± 1.8^{g}	53.5 ± 2.1^{c}

[1]Samples with the same superscript (a-g) within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

In carrot slices blanched in the ultrasonic bath, a higher inactivation of POD for treatment USB 60-30 and USB 60-60 (25.5 and 11.9% of residual activity, respectively) can be observed, as compared with the results obtained for the conventional blanching C60-40 (40.9%), indicating the usefulness of the combined effect of temperature and US for enzyme inactivation.

A noticeable reduction of POD activity with time was observed during US treatments with probe at temperatures lower than 35 °C; values of residual activity close to 60% being reached after 60 min, irrespective of carrot geometry. However, to obtain higher inactivation, the application of US with heat generation was necessary; pre-treatment USP 70-15 providing the highest enzyme inactivation (17.4 and 6.7% residual POD activity in sliced and minced carrots, respectively). In addition, similar results of POD inactivation were obtained for carrots processed by either US (USP 60-10) or by conventional mild temperature treatments (C60-40).

Although it is difficult to exactly determine the effect of sample geometry on enzyme inactivation, the larger specific area would be the main factor to explain the higher inactivation of minced carrots after treatments carried out at high temperature (conventional and US with probe at 60 and 70°C). On the contrary, this factor seems not to be as significant in US blanching treatments carried out in bath, probably due to the previously mentioned formation of aggregates taking place in minced carrots.

With respect to US probe experiments, the combined effect of ultrasonic waves and heat treatment on enzyme inactivation appears to be more effective than US on its own. De Gennaro et al. (1999), in a kinetic study carried out in solution on the inactivation of peroxidase type VI from horseradish, found a considerable reduction in the *D* value when US were applied at 80 °C. According to Cruz et al. (2006), who studied the peroxidase inactivation kinetics in watercress by thermosonication, the reduction of specific activity could be related to the conformation changes in the tertiary structure of the enzyme, and in the three-dimensional structure of the active site affecting the enzyme-substrate interaction.

Total inactivation of PME (**Table 4.22**) was achieved after conventional treatments CS-2, CB-1 and C95-5, whereas heating at 60 °C for 40 min (C60-40) preserved approximately 60% of the enzymatic activity. Similarly,

Lemmens et al. (2009) found 80% of PME residual activity at 60 °C and total enzyme inactivation at 90 °C during the blanching of carrots by microwave, ohmic and conventional heating. Comparing PME results with those of POD shown above, the lower stability of PME at high temperatures was confirmed (Chinnery, 1983; Tijskens et al., 1997; Ni et al., 2005). However, in the case of LTLT treatments (C60-40), the presence of two isoenzymes of PME (bound and free) with different susceptibility to heat, could explain its higher residual activity as compared to POD (Tijskens et al., 1997).

During the US bath assays, no inactivation of PME was detected in USB 40-30 treated carrot samples and 60 min of treatment or higher temperature (60 °C) were needed to achieve a significant reduction of the activity of this enzyme. The application of the experimental setting of **Figure 4.16** (with and without heat generation) did not produce either an important deactivation of PME. Thus, after US treatments, the values of enzymatic residual activity were always within the range 50-80%, and no conclusions derived from the sample geometry and/or processing temperature could be obtained. An additional advantage of US probe is to obtain a higher POD inactivation that with US bath while remain a high activity of PME that can contribute to the textural stability of samples.

Variable results have been reported on the inactivation of PME in tomato juice (Raviyan et al., 2005; Wu et al., 2008; Terefe et al., 2009). In all these cases, the application of US resulted in the reduction of PME activity dependent on the media in which the enzyme was suspended and on the US processing conditions. In addition, previous papers have also shown surprising results during the inactivation of PME by thermal treatment. Thus, in potato, Abu-Ghannam and Crowley (2006) found 60% of residual activity after treatments at 65-90 °C for 5 min and 0% at 80 °C for 10 min, whereas in samples treated at 65 °C for 15 min a 85% of residual activity was detected, probably due to some reactivation effect.

All these results underline the difficulty to identify the mechanism responsible for enzyme deactivation during sonication. Inactivation of enzymes by US is mainly attributed to a mixture of mechanical and chemical effects of cavitation, which are the formation, growth and implosion of bubbles caused by US (Raviyan et al., 2005). The sonochemically generated radicals can oxidise the residues of amino acids such as tryptophan, tyrosine,

hystidine and cysteine that are involved in the catalytic activity and stability of several enzymes. Free radicals have been reported to participate in the ultrasonically-induced inactivation of horseradish peroxidase and catalase, among other enzymes (Terefe et al., 2009). Moreover, US efficacy is dependent upon numerous extrinsic and intrinsic operating parameters (O´Donnell et al., 2010).

Effects of ultrasound and conventional blanching on total soluble solids and carbohydrates

Fructose, glucose and sucrose were the major carbohydrates in all the blanched samples analysed, regardless of the blanching treatment applied. Minor carbohydrates such as *scyllo*-inositol, *myo*-inositol and sedoheptulose were also present in all the samples under study.

Tables 4.23 and **4.24** list, respectively, the loss of total soluble solids and of low-molecular-weight carbohydrates due to leaching during the blanching of carrots by conventional and US treatments. As expected, the losses of total soluble solids were higher in minced over sliced carrots since the surface:volume ratio is 2-fold higher in the former. For both types of geometry, blanching treatment CS-2 provided the lowest loss of total soluble solids and carbohydrates in carrot samples. With the exception of CS-2 and CB-1 samples, all carrots presented a slight decrease in the pH values of the blanching water (results not shown). This could probably be due to the fact that, under these conditions, a higher amount of organic acids could be transferred to water by carrot leaching (Cruz et al., 2007).

With respect to major low-molecular-weight carbohydrates, glucose and fructose were the main lost carbohydrates, followed by sucrose, probably due to the higher diffusivity and solubility of monosaccharides as compared to sucrose (Weast, 1980). Machewad et al. (2003) reported total soluble sugar losses of 62.5% in the conventional blanching of carrots carried out in boiling water for 5 min, whereas Nyman et al. (2005) found 24 and 38% losses of soluble solids and carbohydrates, respectively, in carrots blanched in boiling water for 7 min. All these differences might be attributed, among other factors, to the different sample geometry and water/sample ratio used in the reported studies.

Table 4.23 Loss of total soluble solids by leaching determined in the blanching water of carrot samples submitted to different conventional and US treatments. Mean of two replicates ± standard deviation

	Leaching loss (%)	
Samples	***Minced***	***Sliced***
CS-2	0.7 ± 0.3[a1]	0.6 ± 0.3[a]
CB-1	11.9 ± 0.2[b]	9.6 ± 0.9[b]
C95-5	31.4 ± 0.2[c]	19.2 ± 0.9[c]
C60-40	26.5 ± 3.9[d]	15.2 ± 0.6[d]
USB 40-30	7.1 ± 0.2[e]	3.2 ± 1.0[ae]
USB 40-60	36.6 ± 4.6[f]	15.0 ± 0.4[d]
USB 60-30	48.5 ± 0.1[g]	24.2 ± 1.0[f]
USB 60-60	52.4 ± 0.1[g]	37.7 ± 5.8[g]
USP 35-15	6.2 ± 2.1[e]	3.1 ± 0.6[ae]
USP 35-60	13.5 ± 0.3[b]	6.3 ± 0.5[be]
USP 60-10	26.4 ± 0.1[d]	19.1 ± 0.2[c]
USP 70-15	37.4 ± 1.1[f]	35.6 ± 0.0[g]

[1]Samples with the same superscript (a-g) within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Minor carbohydrates were lost in variable amounts depending on the carbohydrate and the assayed treatment. The most striking result was the high leaching loss of sedoheptulose for any of the blanching treatments evaluated with values in the range 18-66%, higher than those obtained for *scyllo*-inositol (0-54%) and *myo*-inositol (0-57%).

Table 4.24 Loss (%) of major, minor and total carbohydrates in carrot samples blanched under different conventional and US treatments. Mean of two replicates ± SD

Sample	Fructose[1]		Glucose		Sucrose		*Scyllo*-inositol		*Myo*-inositol		Sedoheptulose		Total carbohydrates	
	Minced	Sliced	Minced	Sliced	Minced	Sliced	Minced	Sliced	Minced	Sliced	Minced	Sliced	Minced	Sliced
CS-2	0.4±0.2^{a}	1.3±0.0^{a}	0.3±0.1^{a}	0.1±0.0^{a}	0.3±0.1^{a}	0.3±0.0^{a}	0.3±0.0^{a}	0.0±0.0^{a}	0.4±0.1^{a}	2.9±0.0^{a}	18.4±0.3^{a}	18.3±0.0^{a}	1.3±0.4^{a}	1.2±0.3^{a}
CB-1	23.5±1.4^{b}	9.6±0.1^{bc}	22.6±0.9^{b}	10.7±0.4^{bc}	14.3±1.7^{b}	10.5±0.6^{b}	17.2±2.2^{bc}	14.2±1.4^{bcd}	18.8±0.8^{b}	19.4±0.7^{b}	34.7±0.9^{b}	30.5±0.9^{b}	18.0±1.5^{b}	11.6±0.5^{b}
C95-5	48.0±1.3^{c}	28.2±0.4^{d}	48.0±1.8^{c}	27.7±0.9^{d}	40.2±6.3^{c}	20.6±1.6^{c}	32.7±3.5^{d}	27.9±1.2^{e}	42.6±5.5^{cd}	29.9±0.6^{c}	51.6±0.2^{c}	41.5±0.9^{c}	43.1±4.6^{c}	23.9±0.7^{c}
C60-40	44.6±3.7^{cd}	30.4±5.3^{d}	45.0±2.2^{c}	28.9±5.8^{d}	31.0±4.7^{d}	22.5±5.3^{c}	39.5±4.1^{de}	28.8±7.1^{e}	39.0±4.1^{cd}	31.8±8.2^{c}	47.8±6.3^{c}	43.4±5.4^{cd}	36.1±4.2^{d}	25.8±4.5^{c}
USB 40-30	19.5±2.7^{be}	3.6±1.0^{ab}	12.5±0.9^{de}	2.5±1.5^{a}	5.3±0.9^{ae}	2.9±1.2^{a}	20.7±6.4^{c}	3.2±0.6^{a}	13.5±5.3^{be}	4.7±1.3^{a}	33.3±6.2^{b}	21.5±1.2^{a}	10.0±1.4^{e}	4.0±1.2^{a}
USB 40-60	35.1±6.0^{f}	21.7±3.6^{e}	29.7±6.5^{f}	20.6±4.3^{e}	28.5±6.8^{d}	10.7±0.5^{b}	47.6±5.2^{ef}	14.6±6.0^{cd}	45.8±2.7^{d}	19.1±7.9^{b}	46.2±5.5^{c}	37.1±6.0^{bc}	30.7±6.5^{d}	15.3±1.9^{b}
USB 60-30	58.0±3.3^{g}	46.5±4.1^{f}	58.1±3.4^{g}	46.2±4.8^{f}	47.6±3.9^{f}	37.6±5.0^{d}	53.7±3.9^{f}	39.2±8.3^{f}	57.3±7.0^{f}	33.7±1.7^{cd}	63.8±2.9^{d}	48.9±1.8^{de}	51.6±3.8^{fg}	40.8±2.0^{d}
USB 60-60	56.5±3.2^{g}	53.9±6.8^{g}	57.9±2.6^{g}	56.0±7.1^{g}	56.4±1.3^{g}	40.8±5.3^{d}	52.1±4.7^{f}	44.1±6.7^{f}	55.8±0.7^{f}	54.4±6.3^{e}	65.9±5.3^{d}	64.2±7.0^{f}	57.1±0.3^{g}	46.4±5.9^{e}
USP 35-15	8.7±1.3^{h}	4.5±2.1^{ab}	8.7±1.8^{d}	4.6±1.7^{ab}	7.2±2.5^{abe}	1.6±1.1^{a}	8.6±1.1^{ab}	3.4±0.1^{a}	10.4±0.1^{e}	3.5±0.3^{a}	24.7±2.1^{ae}	20.0±0.7^{a}	8.6±1.9^{e}	3.5±0.2^{a}
USP 35-60	18.1±3.0^{be}	5.1±1.3^{ab}	16.9±2.6^{be}	4.4±1.4^{ab}	9.2±0.1^{be}	4.8±1.9^{ae}	14.7±0.7^{bc}	5.1±1.2^{abc}	16.4±0.7^{be}	6.5±1.8^{a}	32.2±2.6^{be}	22.8±2.1^{a}	13.0±1.0^{be}	5.8±1.8^{a}
USP 60-10	40.9±5.6^{df}	13.4±0.3^{c}	43.0±4.9^{c}	13.8±0.2^{ce}	25.6±3.5^{d}	8.4±0.3^{be}	31.1±8.1^{d}	23.8±2.1^{de}	35.4±3.0^{c}	16.9±0.3^{b}	50.7±2.3^{c}	31.6±0.1^{b}	31.9±0.5^{d}	11.3±0.3^{b}
USP 70-15	56.9±0.1^{g}	49.6±1.1^{fg}	56.3±0.2^{g}	47.5±0.7^{f}	45.1±0.1^{cf}	25.1±0.6^{c}	42.3±1.6^{e}	45.5±9.2^{f}	54.6±1.2^{f}	41.7±3.4^{d}	64.7±0.2^{d}	54.0±4.5^{e}	49.6±0.1^{f}	33.8±0.7^{f}

[1]Samples with the same superscript (a-h) within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Regarding samples processed by US pre-treatments, the main losses were detected when samples were treated with generation of heat for longer times. For US bath and US probe blanching treatments carried out at low temperatures (USB 40-30 and USP 35-15), very low losses of total soluble solids (3-7%) and carbohydrates (3-10%) were found. In general, higher sugar losses were observed by other authors for papayas (13.8%), banana (21.3%), pineapples (23.2%) and Malay apples (17%) after 30 min treatment at 30 °C in an ultrasonic bath of 45 kHz (Fernandes & Rodrigues, 2007; Rodrigues & Fernandes, 2007; Fernandes et al., 2008a; Rodrigues et al., 2009b). These differences could be due to the different susceptibility of vegetable substrates to the effects of US. In the assays with US probe, taking into account only the effect of US (USP 35-15 and USP 35-60), the total soluble losses were low even after 60 min (< 6.5% in slices). However, higher losses were observed after treatments carried out at a final temperature of 60 or 70 °C, with values close to 37% in the latter. Finally, USP 60-10 gave rise to similar losses of total soluble solids and carbohydrates than conventional blanching at mild temperature (C60-40); particularly for minced carrot samples.

Conclusions

This work presents preliminary results on the efficiency of different conventional and US treatments for blanching of carrots. Although further research on additional indicators would be necessary to draw definite conclusions, it seems that US for blanching purposes is more convenient with probe and heat generation. According to the obtained results, among the US treatments of carrot samples assayed, those carried out at temperatures up to 70 °C gave rise to the highest enzymatic deactivation (90 and 50% POD and PME inactivation, respectively), with losses of total soluble solids ~ 37% and up to almost 50% of total carbohydrates. Moreover, US blanching with probe at temperatures up to 60 °C for 10 min presented similar values of enzyme inactivation and similar losses by leaching than the conventional treatment at 60 °C for 40 min. Therefore, the application of US for carrot blanching, under these conditions, could constitute an adequate treatment

with similar effects to LTLT conventional blanching but with a noticeable reduction of time. These treatments could also be considered as an advantageous for those applications in which partial inactivation of PME is required for better preservation of carrot structure. The results obtained in this work may contribute to broaden the application of US as an effective procedure for blanching of vegetables, particularly under mild conditions.

4.2.1.2 Cambios químicos, físicos y sensoriales durante la deshidratación

4.2.1.2.1 Parámetros de calidad en zanahorias deshidratadas por convección previamente escaldadas por ultrasonidos y convencionalmente

Quality parameters in convective dehydrated carrots blanched by US and conventional treatment

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Food Chemistry (2013), doi: 10.1016/j.foodchem.2013.03.028

Abstract

The effect of previous US and conventional blanching treatments on drying and quality parameters (2-FM-AA -as indicators of lysine and arginine participation in the Maillard reaction-, carbohydrates, total polyphenols, protein profile, rehydration ratio, microstructure changes) of convective dehydrated carrots has been assessed. The most striking feature was the influence of blanching on the subsequent 2-FM-AA formation during drying, probably due to changes in the protein structure. The highest values of 2-FM-AA were found in carrots conventionally blanched with water at 95 ºC for 5 min. However, samples previously treated by US presented intermediate values of 2-FM-AA and carbohydrates as compared to the conventionally blanched samples. Dried carrots previously subjected to US blanching preserved their total polyphenol content and showed rehydration properties, which were even better than those of the freeze-dried control sample. The results obtained here underline the usefulness of 2-FM-AA as indicators of the damage suffered by carrots during their blanching and subsequent drying.

Introduction

As pointed out by different epidemiological studies, the risk of suffering several degenerative pathologies, such as cancer and cardiovascular diseases, can be decreased with a high intake of vegetables (Liu et al., 2000; Riboli & Norat, 2003). In this sense, their high contents of β-carotene, vitamins C, B1, B2, B6 and B12, folic acid, potassium, magnesium and pectin make carrots (*Daucus carota* L.) one of the healthiest vegetables (Erenturk & Erenturk, 2007). However, as with the rest of vegetables, carrots are highly seasonal and abundantly available at particular times of the year. For extending the availability of this root, several preservation processes have been assayed. Among them, drying is one of the most important since it not only significantly extends vegetable shelf-life but also diversifies the offer of foods for consumers (Lewicki, 1998a).

The most common dehydration technique used in the vegetable industry is hot air drying under forced convection since it offers the advantages of low complexity and cost (Garcia-Noguera et al., 2010). Several studies have been performed on the drying of carrots; modeling of the process was one of the most important aspects studied (Erenturk & Erenturk, 2007; Mulet et al., 1989). However, convective drying can also give rise to significant chemical changes (non-enzymatic browning, among others), which may affect the quality of the product. Most of the browning occurring during drying and subsequent storage is via the Maillard reaction (MR) (Mcbean et al., 1971). In this sense, the usefulness has been recently demonstrated of 2-FM-AA derivatives and, particularly of furosine (2-furoylmethyl Lys), as sensitive indicators for early detection of MR advance in carrots subjected to drying before important changes in nutritive value can be produced (Rufián-Henares et al., 2008; Soria et al., 2009b; Soria et al., 2010; Wellner et al., 2011).

Moreover, the microstructure of vegetables might also be damaged during drying. Thus, the loss of integrity of the cell membranes, loss of turgor and deterioration of cell wall structure might result in significant shrinkage and loss of the rehydration potential of dehydrated vegetables (Lewicki, 1998b).

The quality of dried products is not only affected by the drying conditions but also by other operations such as the pre-treatment of the

material (Negi & Roy, 2001). Blanching can reduce the initial number of microorganisms, inactivate enzymes, remove gases from surface and intercellular spaces to prevent oxidation and reduce drying time (Rahman & Perera, 1999). Typically, blanching is carried out by treating the vegetable with steam or hot water for 1-10 min at 75-95 °C; the time/temperature combination selected is dependent on the type of vegetable. In the case of carrots, low-temperature/long-time and high-temperature/short-time blanching methods have been applied (Sanjuán et al., 2005; Shivhare et al., 2009).

In addition, other methodologies such as power US have emerged as an alternative pre-treatment, increasing the mass transfer rate during drying. A number of works have been carried out on the application of US before conventional drying and as a medium to assist osmotic dehydration of vegetables and fruits (Jambrak et al., 2007a; Opalic et al., 2009; Azoubel et al., 2010; Fernandes et al., 2011; Rawson et al., 2011). Most of these works have been carried out in ultrasonic baths at mild temperatures and have been mainly focused on the kinetic of moisture loss during drying: US showed a noticeable reduction in the overall drying time and gave rise to a variable loss of total sugars. In carrots, our research group (Gamboa-Santos et al., 2012a; Gamboa-Santos et al., 2013b), has studied the inactivation of POD and PME, the losses of soluble compounds by leaching and the sensorial properties of dehydrated carrots blanched conventionally or by US (in a bath or with probe treatments). In the present paper, the effect of different blanching (US and conventional) processes on the kinetic of drying and quality of carrots dehydrated in a convective drying prototype system has been investigated, paying special attention to the influence of blanching on the MR evolution during the subsequent drying process. In addition, other complementary quality parameters such as total polyphenols, carbohydrates, proteins, rehydration capacity and microstructural changes have been studied.

Materials and methods

Sample preparation

Fresh carrots (*Daucus carota*L. var. Nantesa) were purchased from a local market in Madrid (Spain) and stored in the dark at 4 °C for a maximum period of 5 days until processing. Carrots were washed in tap water and then were cut into 24 mm diameter slices and 4 mm thick or as minced carrots (1–2 mm).

Processing

In a previous paper (Gamboa-Santos et al., 2012a), a wide range of blanching conditions by conventional or US treatments were assayed. Among them, we selected for the present paper those providing a high enzymatic inactivation of POD and a relatively low loss by leaching.

Table 4.25 summarises the codes and blanching conditions of the samples under analysis in the present paper. In the US assays, an ultrasonic system (450 Digital Sonifier, Branson Ultrasonics Corporation, Danbury, CT, USA) equipped with a temperature sensor and a 13 mm diameter tip directly attached to a disruptor horn (20 kHz, 400 W full power) was used. For steam blanching treatments, an autoclave (CERTOCLAV CV-EL GS, Austria) was used. The carrot-distilled water ratio (40 g: 200 mL) was the same for all carrot pre-treatments assayed.

Table 4.25 Processing conditions used during conventional/US blanching of carrots and further drying by convection at 46 °C and at a drying rate of 4.9 m/s

Sample code	Carrot geometry	Blanching conditions	Drying time(h)
D-CS-2-M	Minced	Steam (98 ºC, 2 min)	7
D-CS-2-S	Sliced	Steam (98 ºC, 2 min)	9
D-CB-1-M	Minced	Boiling water (98 ºC, 1 min)	7
D-CB-1-S	Sliced	Boiling water (98 ºC, 1 min)	9
D-C95-5-M	Minced	Hot water (95 ºC, 5 min)	7
D-C95-5-S	Sliced	Hot water (95 ºC, 5 min)	9
D-C60-40-M	Minced	Hot water (60 ºC, 40 min)	7
D-USP60-10-M	Minced	US probe (up to 60 ºC, 10 min)	7
D-USP70-15-S	Sliced	US probe (up to 70 ºC, 15 min)	9

Blanched carrots were subsequently dried by convection in a tray dryer (SBANC, Edibon Technical Teaching Units, Spain) at a temperature of 46 °C and an air rate of 4.9 m/s. These operating conditions had previously been optimized by Gamboa-Santos et al. (2012b) on the basis of the drying kinetic and the levels of quality parameters such as the 2-FM-AA determined in carrots subjected to different convective drying conditions. For comparative purposes, a previously freeze-dried (FD) sliced raw carrot was used as control.

Analytical determinations

Characterization of samples

Water activity (a_w) was determined at 25 °C using a Novasina a_w Sprint TH-500 (Pfäffikon, Switzerland) system previously calibrated with saturated solutions of different salts. Total nitrogen (TN) was determined by means of the Kjeldahl method, and the protein level was calculated using 6.25 as conversion factor (TN × 6.25) (AOAC, 1990b). The dry matter (DM) content was determined gravimetrically by drying the samples to constant weight (AOAC, 1990a). All determinations were carried out in duplicate, and the results expressed as mean values.

Extraction and analysis of total phenolic content (TPC)

Aliquots (0.1 g) of dried carrot samples were homogenized in 2.5 mL of HPLC grade methanol by using an Ultra Turrax (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) operating at 24,000 rpm for 1 min. During the extraction, the temperature was controlled by using an ice-water bath. Homogenates were stirred (750 rpm) for 20 min at room temperature using a Thermomixer (Eppendorf, Germany) and centrifuged at 2,000*g* for 15 min. Supernatants were filtered through PVDF Acrodisc syringe filters (0.45 μm, Sigma-Aldrich) for subsequent analysis.

TPC content of carrot extracts was colorimetrically determined using Folin–Ciocalteu reagent (2 N, Sigma), as described by Singleton, Orthofer and Lamuela-Raventos (1999), with slight modifications. The filtered methanolic solution (100 μL), added with 100 μL of MeOH, 100 μL of Folin-

Ciocalteu reagent and 700 µL of 75 g/L Na_2CO_3 was vortexed briefly. The samples were left in the dark for 20 min at room temperature. Following this, the samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the sample was read at 735 nm in a spectrophotometer (Power Wave XS Microplate, BIO-TEK) using the KC Junior Data Reduction software. Aqueous solutions of gallic acid (Sigma-Aldrich) in the range 10-400 mg/L were used to prepare the calibration curve. Results (average for n = 3 replicates) were expressed as milligrams of gallic acid equivalent (GAE)/g DM of carrots.

GC analysis of soluble carbohydrates

Soluble carbohydrates were determined by GC-FID following the method of Soria et al. (2010). Samples were ground to powders using a laboratory mill IKA A-10 (IKA Labortechnik, Staufen, Germany) and aliquots of 30 mg were weighed into a polyethylene tube and extracted at room temperature with 2 mL of Milli-Q water under constant stirring for 20 min. Next, 8 mL of absolute ethanol were added, followed by 0.2 mL of an ethanolic solution 10 mg/mL of phenyl-β-D-glucoside (Sigma-Aldrich Chemical, St. Louis, Missouri, USA) used as internal standard. After stirring for 10 min, samples were centrifuged at 10 °C and 9,600*g* for 10 min and the supernatant was collected. Precipitates were subjected to a second extraction with 10 mL of 80% ethanol under the same conditions to obtain recovery values close to 100%. Finally, an aliquot (2 mL) of supernatant was evaporated under vacuum at 40 °C and derivatised.

The dried mixtures were treated with hydroxylamine chloride (2.5%) in pyridine (200 µL) and kept at 70 °C for 30 min. Subsequently, samples were persilylated by addition of 200 µL of hexamethyldisilazane and 20 µL of trifluoroacetic acid, followed by heating at 50 °C for 30 min. Reaction mixtures were centrifuged at 8,800*g* for 2 min and supernatants containing the derivatised sugars were injected into the GC or stored at 4 °C until analysis.

The trimethylsilyloximes of carbohydrates were quantitatively analysed (n = 3) in an Agilent Technologies 7890A gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with an HP-5MS capillary column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness) (J &

W Scientific, Folsom, California, USA). Nitrogen at a flow rate of 1 mL/min was used as carrier gas. The oven temperature was held at 200 °C for 11 min, raised to 270 °C at a heating rate of 15 °C/min and raised again to 315 °C at 3 °C/min. Temperatures of the injector and the flame ionization detector were 280 °C and 315 °C, respectively. Injections were carried out in split mode (1:30). Data acquisition and integration were performed using Agilent ChemStation Rev. B.03.01 software (Wilmington, DE). Solutions containing fructose, glucose, *myo*-inositol and sucrose were prepared over the expected concentration range in carrot samples to calculate the response factor of each of these sugars relative to the internal standard.

Confirmation of identities was done based on experimental data for standards (linear retention indices and mass spectra) and data from literature (Soria et al., 2009a). GC-MS analyses of derivatised samples were carried out using a 7890A gas chromatograph coupled to a 5975C quadrupole mass detector (both from Agilent Technologies, Palo Alto, CA, USA). Chromatographic conditions other than carrier gas (He) were similar to those previously mentioned for GC-FID analysis. The mass spectrometer was operated in electron impact mode at 70 eV, scanning the 35-700 m/z range. Acquisition was done using HP ChemStation software (Agilent Technologies).

2-FM-AA determination

Samples of dehydrated carrots (0.25 g) were thermally hydrolysed under inert conditions (helium) with 4 mL of 8 N HCl at 110 °C for 23 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolysed samples were filtered through a Whatman No. 40 paper filter and 0.5 mL of the filtrate was applied to a Sep-Pack C_{18} cartridge (Millipore) prewetted with 5 mL of methanol and 10 mL of water and then eluted with 3 mL of 3 N HCl.

Determination of 2-FM-AA were carried out by ion-pair RP-HPLC analysis (Resmini & Pellegrino, 1991), using a C_8 column (250 mm × 4.6 mm i.d.) (Alltech, Lexington, KY) thermostated at 37 °C, with a linear binary gradient composed of phase A (4 mL/L acetic acid) and phase B (3 g/L KCl in phase A solution). The elution program was as follows: 0-12 min: 100% A; 20-22.5 min: 50% A; 24.5-30 min: 100% A. The flow rate was 1.2 mL/min and injection (50 µL) was carried out using a manual Rheodyne valve. Detection

was done in a variable-wavelength detector (LCD Analytical SM 4000) set at 280 nm.

Quantitation was performed by the external standard method, using a commercial standard of furosine (Neosystem Laboratoire, Strasbourg, France). All analyses were done in triplicate and mean values expressed as milligrams per 100 g of protein.

Protein profile by sodium dodecyl sulphate-polyacrylamide gel electrophoresis *(SDS-PAGE)*

Powdered dehydrated carrot samples (100 mg) were mixed with 2 mL of 1% sodium metabisulfite (Merck, Darmstadt, Germany) aqueous solution. Next, samples were stirred thoroughly for 2 h and centrifugated at 3,000*g* for 15 min. The supernatants were analysed by SDS-PAGE.

Protein analysis was carried out by adding 32.5 µL of sample supernatant to 12.5 µL of 4 x NuPAGE LSD sample buffer (Invitrogen, Carlsbad, California, USA) provided with 5 µL of 0.5 mol/L dithiothreitol (Sigma-Aldrich). Samples were heated at 70 °C for 10 min and 20 µL were loaded on a 12% polyacrylamide NuPAGENoveBis-Tris precast gel (Invitrogen). Gels were run for 41 min at 120 mA per gel and 200 V with a continuous MES SDS running buffer (Invitrogen) and were stained using the Colloidal Blue Staining Kit (Invitrogen). A mixture of standard proteins with molecular weights ranging from 2.5 to 200 kDa (Invitrogen) was used to estimate the molecular weight of carrot proteins. Myosin, 200 kDa; β-galactosidase, 116.3 kDa; phosphorylase B, 97.4 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55.4 kDa; lactate dehydrogenase, 36.5 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa; aprotinin, 6 kDa; insulin B chain, 3.5 kDa and insulin A chain, 2.5 kDa were chosen as standards.

Rehydration ratio (RR)

Rehydration of dehydrated carrot samples was performed according to Soria et al. (2010). Dried samples were rehydrated by immersion in distilled water (solid: liquid ratio of 1:50) at ambient temperature for 24 h. Carrots were placed on paper towels to remove the surface water and then weighted.

Each rehydration experiment was performed in triplicate and RR was calculated as:

$$RR = m_r/m_d \quad (1)$$

where m_r and m_d are the weights of rehydrated and dehydrated carrot, respectively.

Scanning electron microscopy (*SEM*)

The surface microstructure of dehydrated or control samples was observed by scanning electron microscopy. Prior to SEM observations, the samples were coated with gold: palladium 80:20 in a sputter coater SC7460 Polaron (Quorum Technologies, Newhaven, UK), at 5-10 mA and 800 V plasma current in order to stabilize the structure. Then they were viewed with a Philips XL 30 ESEM Electron Microscope at an accelerating voltage of 25 kV. Duplicate specimens were viewed at different magnifications (200, 400, 800 and 1500) and images of representative areas were saved for further analysis.

Statistical analysis

To study the effect of temperature and air rate on the quality parameters determined, one-way analyses of variance (ANOVA) were carried out using Statgraphics (version 5.1; StatPoint, Inc., Warrenton, VI, USA). Individual treatments were compared using the least significant difference test (LSD, 95%).

Results and discussion

Dehydration of blanched carrot samples

Figure 4.17 depicts the drying curves obtained in the dehydration of minced and sliced carrots by convection after different blanching treatments (see **Table 4.25**). As can be observed, curves with different slopes were obtained depending on the blanching applied and the geometry of samples; minced carrots presented higher slope values (0.059-0.221) than sliced

carrots (0.040-0.082). This fact could be due to the higher values of initial moisture of minced (7.6-24.0 kg H_2O/kg DM) as compared to sliced carrots (6.9-13.3 kg H_2O/kg DM) and/or the higher specific surface of minced carrots. Thus, for boiling blanched samples, with similar initial moisture, minced carrot samples were dehydrated more quickly than sliced ones. Moreover, carrots blanched by conventional treatments at 60 °C for 40 min presented the highest slope value and the highest initial moisture content.

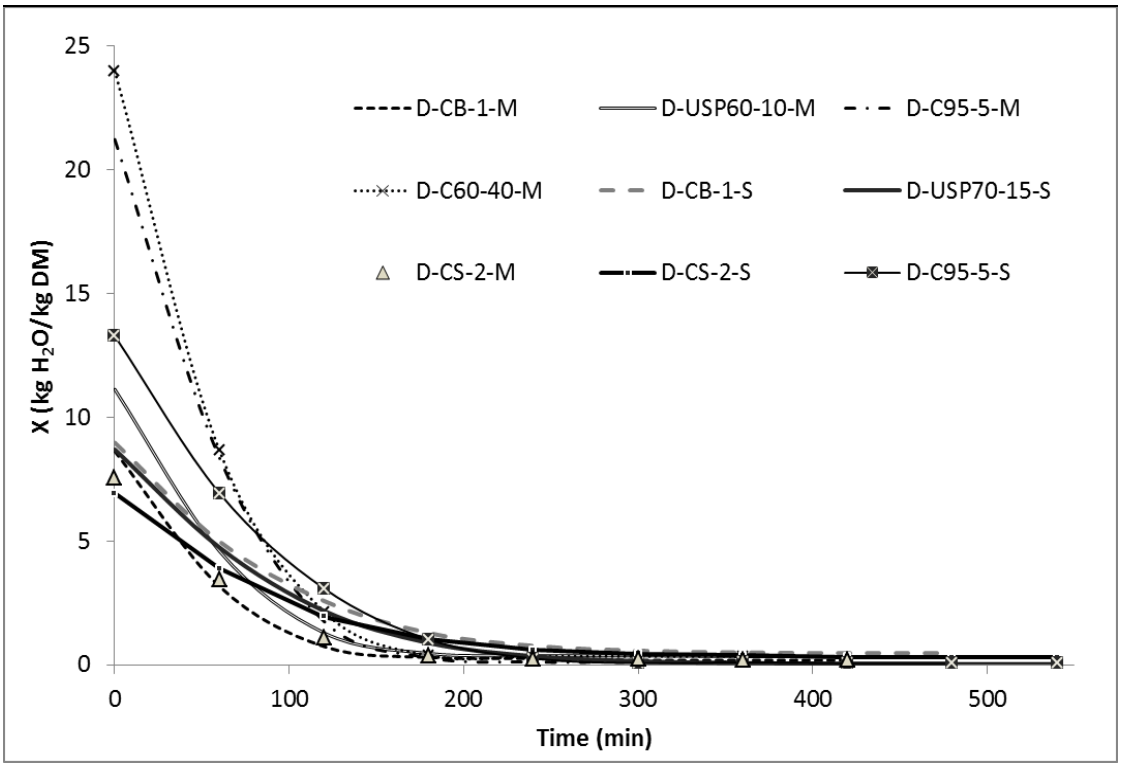

Figure 4.17 Drying curves obtained in the dehydration by convection at 46 °C and at a drying rate of 4.9 m/s of minced and sliced carrots subjected to different blanching treatments (Table 4.25).

In relation to the final product, dried samples showed DM contents in ranges from 88.5-93.1% and 85.0-88.7%, respectively, for minced and sliced carrots. All these values were very close to those considered as microbiologically safe for dried products (85%) (Belitz et al., 2009a). Determination was also made of a_w and the values obtained were within the interval from 0.238 to 0.375. As is known, foods with a_w values near 0.3 are stable against non-enzymatic browning, microorganism development and enzymatic activities during their adequate storage (Labuza, 1971). In addition, samples that after blanching presented some residual activity of

POD (subjected to steam blanching, hot water at 60 °C for 40 min and to US blanching at 60 °C for 10 min and at 70 °C for 15 min (Gamboa-Santos et al., 2012a) were evaluated after drying and, in all cases, no residual activity of this enzyme was found. Thus, regardless of the blanching treatment applied, all the dried carrots under study showed great stability, which might guarantee their safe consumption over the course of their shelf-life.

The dehydration of samples pre-treated with US originated final products with intermediate slopes, as shown in **Figure 4.17**. Other authors have found that different fruits (Malay apple, melon, pineapple) subjected to US pre-treatment dried faster during the air-drying stage compared to fresh fruit with no pre-treatment. This could be explained in that US pre-treatment might increase the effective water diffusivity in the fruit, thereby reducing the dehydration time (Fernandes et al., 2011; Mothibe et al., 2011).

Chemical changes during drying of carrot samples

TPC values of samples dried by convection after several blanching procedures (**Table 4.26**) were within the 1.312-1.524 mg GAE/g DM range. These results were similar to those published by Soria et al. (2010) for sliced carrots of the same size and blanched with boiling water for 1 min and further dehydrated by US-assisted convective drying. A slight decrease, only significant for several samples, was observed in the dried carrots previously blanched by conventional heat treatments as compared to the control sample. It has been described that changes in physical properties of carrots processed under different drying conditions can modify the extractability of bioactive compounds (Gorinstein et al., 2009). Thus, the freeze-drying process might alter tissue structure and make the extraction of flavonoids easier (Pérez-Gregorio et al., 2011). It is also noteworthy that samples subjected to a previous blanching by US presented similar TPC values to those of FD carrot samples. This could be due to the fact that US treatment can give rise to pores in the vegetal tissue and, consequently, improve the extraction of polyphenols during sample preparation. In spite of the small differences observed, in general, it is possible to say that hardly any change in the TPC content, and indirectly in their antioxidant activity, was measured in the samples analysed. Previous papers have demonstrated a high

correlation between TPC and antioxidant activity measured by the ORAC method and that dehydration might be considered a good method for preserving the content of these compounds (Rababah et al., 2005; Soria et al., 2010).

Table 4.26 Total Phenolic Content (TPC) and 2-FM-AA amount (mg/100 g protein) determined in dehydrated carrot samples previously subjected to different blanching treatments (mean of three replicates ± SD)

Carrot samples	TPC (mg GAE/g DM)	2-FM-Lys + 2-FM-Arg (mg/100 g protein)
FD (control)	1.541 ± 0.021^{bca}	-
D-CS-2-M	1.367 ± 0.025^{ab}	159.1 ± 2.3^{a}
D-CS-2-S	1.329 ± 0.023^{a}	152.1 ± 0.0^{a}
D-CB-1-M	1.373 ± 0.068^{ab}	139.5 ± 8.6^{a}
D-CB-1-S	1.342 ± 0.140^{a}	117.58 ± 3.4^{a}
D-C95-5-M	1.312 ± 0.001^{a}	660.7 ± 15.3^{b}
D-C95-5-S	1.382 ± 0.026^{ab}	681.5 ± 26.6^{b}
D-C60-40-M	1.352 ± 0.054^{a}	104.3 ± 6.6^{a}
D-USP60-10-M	1.434 ± 0.055^{c}	274.4 ± 26.8^{c}
D-USP70-15-S	1.524 ± 0.028^{abc}	342.7 ± 13.31^{d}

[a]Samples with the same superscript letter (a-d) within the same column showed no statistically significant differences for their mean values at the 95% confidence level.

Other changes that can take place during dehydration of vegetables are the losses of carbohydrates due to thermal treatment and/or leaching during blanching (Rodríguez-Sevilla et al., 1999; Wennberg et al., 2006). **Table 4.27** shows the carbohydrate content of dried carrots previously subjected to the various blanching procedures assayed. Fructose, glucose and sucrose were the major carbohydrates determined in all the samples analysed; sedoheptulose, *scyllo*- and *myo*-inositol were also present as minor carbohydrates in all these samples. In general, carbohydrate content was in good agreement with data previously reported for raw and processed carrots (Soria et al., 2009a, 2010; Gamboa-Santos et al., 2012a).

Table 4.27 Quantitative analysis of carbohydrates in dehydrated carrots under analysis (mean of three replicates ± SD)

Samples	Carbohydrates (mg/g DM ± SD)						
	Fructose	**Glucose**	**Sucrose**	***Scyllo*-inositol**	***Myo*-inositol**	**Sedoheptulose**	**Total**
FD	67.27±2.68$^{a[1]}$	73.99±2.94^{a}	449.50±5.5^{a}	1.48±0.02^{a}	4.76±0.14^{a}	2.56±0.02^{a}	608.63±11.56^{a}
D-CS-2-M	67.26±0.00^{a}	73.97±0.00^{a}	449.05±0.02^{a}	1.48±0.00^{a}	4.76±0.00^{a}	2.56±0.00^{a}	603.80±0.01^{a} (0.8%)[2]
D-CS-2-S	67.19±0.00^{a}	73.90±0.02^{a}	448.65±0.00^{a}	1.48±0.00^{a}	4.74±0.00^{a}	2.55±0.00^{a}	603.14±0.01^{a} (0.9%)
D-CB-1-M	41.15±0.30^{c}	46.05±0.16^{e}	377.58±0.99^{d}	1.03±0.04^{d}	3.30±0.08^{c}	1.89±0.02^{d}	495.00±1.68^{d} (18.7%)
D-CB-1-S	57.39±0.07^{b}	62.63±0.02^{b}	409.36±1.83^{b}	1.22±0.01^{b}	3.97±0.01^{b}	2.09±0.02^{b}	546.24±2.5^{b} (10.2%)
D-C95-5-M	31.13±0.51^{d}	34.81±0.23^{d}	283.20±2.15^{e}	0.86±0.11^{e}	2.53±0.04^{e}	1.32±0.01^{e}	304.91±2.72^{e} (49.9%)
D-C95-5-S	39.00±1.16^{c}	41.80±1.31^{c}	349.53±2.48^{c}	1.00±0.02^{cd}	3.20±0.06^{c}	1.51±0.04^{c}	401.57±4.00^{c} (34.0%)
D-C60-40-M	34.37±0.62^{cd}	42.00±3.19^{c}	311.33±11.68^{e}	0.57±0.06^{f}	2.67±0.04^{de}	1.46±0.01^{c}	392.9±17.56^{c} (35.5%)
D-US60-10-M	39.27±1.08^{c}	40.88±3.28^{c}	343.04±23.77^{c}	0.91±0.06^{ce}	2.92±0.25^{d}	1.66±0.19^{f}	435.29±29.45^{f} (28.5%)
D-US70-15-S	31.76±0.88^{d}	35.42±1.18^{d}	333.88±1.20^{c}	0.86±0.04^{e}	2.81±0.04^{d}	1.47±0.02^{c}	404.46±1.64^{c} (33.5%)

[1]Samples with the same superscript letter (a-f) within the same column showed no statistically significant differences for their mean values at the 95% confidence level.
[2]In brackets, losses of total carbohydrates by lixiviation during blanching with respect to FD sample (control).

As observed in **Table 4.27**, the concentration of carbohydrates in dried carrot samples previously steam blanched (D-CS-2-M and D-CS-2-S) showed no significant differences with respect to FD sample. However, in the other type of samples, significant ($p < 0.05$) losses (10.2-49.9% total carbohydrates) were detected in relation to the same control sample. When considering the same blanching conditions, sliced carrots preserved carbohydrate content better, probably due to the lower specific surface as compared to minced ones. The lowest amount of carbohydrates was detected in dried samples subjected to a previous conventional blanching at 95 and 60°C. With respect to dried samples pre-treated by US, the carbohydrate content was close to that of some conventional blanching treatments.

Regardless of the geometry of the sample, the loss of fructose and glucose was higher than that of sucrose, probably due to the higher solubility of monosaccharides as compared to sucrose in the case of losses due to blanching. A certain loss of reducing carbohydrates (fructose and glucose) could also be suspected as a result of their involvement in the MR. However, when comparing the results obtained after drying of samples with those previously reported by Gamboa-Santos et al. (2012a) for carrots subjected to identical blanching conditions (**Table 4.24**), it can be concluded that the major losses of carbohydrates (considering the overall process) take place by lixiviation during blanching. Thus, the operating conditions used here for convective drying (46 °C, 4.9 m/s) seem not to be strong enough to give rise to appreciable changes in the carbohydrate fraction.

As MR mainly takes place under the moisture conditions achieved during the drying process, MR assessment was also carried out in the carrot samples under study by means of the determination of 2-FM-AA (**Table 4.26**). Although, as previously indicated, hardly any change was observed in the fraction of reducing carbohydrates during the dehydration process considerable formation of these compounds was found in the dehydrated carrots subjected to different blanching treatments. As only traces were detected in blanched samples (Gamboa-Santos et al., 2012a), and all carrot samples were dried under the same operating conditions, the evolution of MR in dried samples can be solely attributable to the drying process.

The highest concentrations of 2-FM-AA were determined in carrots previously blanched at 95 °C for 5 min, whereas the samples with the lowest

evolution of MR were those previously blanched by steam, boiling water and hot water at 60 °C. Carrots treated by US before drying presented intermediate values of this quality marker. Considering the effect of geometry, in general, no clear conclusion can be established since, under the same processing (blanching plus drying) conditions, no significant differences were found between minced and sliced samples.

The amounts of 2-FM-AA found in the samples analysed here were, in general, lower than those reported by other authors for carrots dried under convection (Rufián-Henares et al., 2008; Soria et al., 2009b, 2010; Wellner et al., 2011). This could probably be due either to the more intense processing conditions used in previous studies or to the different variety of carrot processed. To the best of our knowledge, no data have been previously reported on the effect of different blanching procedures on the further evolution of MR during drying.

According to these data, it is presumable that some modification during the previous blanching could affect the structure of the protein and the free amino groups of which could be more or less available to react with the carbonyl group of the reducing carbohydrates during drying. Thus, the highest values of 2-FM-AA for D-C95-5-M and D-C95-5-S could be explained assuming that, under these blanching conditions, a certain unfolding of protein by heat treatment takes place and this unfolding makes the reaction with carbohydrates more favourable. Furthermore, and according to several authors (Leslie et al., 1995; Yoo & Lee, 1993), the stability of proteins can be increased by the carbohydrate concentration. Thus, an increase in hydrophobic interactions and hydrophilic properties, due to the formation of protein-sugar complexes, can stabilize the three dimensional structure of proteins, keeping or protecting its functionality. On the other hand, the samples subjected to US blanching showed relatively high 2-FM-AA values. In this case, since the temperatures of the treatments were low (up to 60 and 70 °C), the main influence was probably the physical effect of US related to the opening of hydrophilic parts of amino acids, as shown by Kreŝić et al. (2008). During US treatment of soy protein isolate, an increase in levels of free amino groups was also observed by Mu et al. (2010), who attributed this result to an unfolding of protein and breaking of peptide bonds by hydrolysis.

To gain more insight into possible changes associated with carrot processing, an SDS-PAGE analysis of the protein fraction of carrots under study was carried out (**Figure 4.18**). As observed, most of the samples presented similar electrophoretic bands to those of the protein profile of the freeze-dried carrots previously reported by Soria et al. (2010). However, lanes 5 and 9, corresponding to D-C95-5-M and D-C95-5-S samples, respectively, presented a different pattern with a non-defined protein profile. In this case, a variety of bands with slower electrophoretic mobility and different molecular weight were detected, indicating that, in addition to a possible unfolding, cross-linking and aggregation of proteins also took place. The previously mentioned high 2-FM-AA content of both samples (D-C95-5-M and D-C95-5-S) (**Table 4.26**) also confirms that blanching carried out under these conditions could have changed the structure of proteins to promote, at a higher extent over other blanching conditions, the evolution of MR during drying.

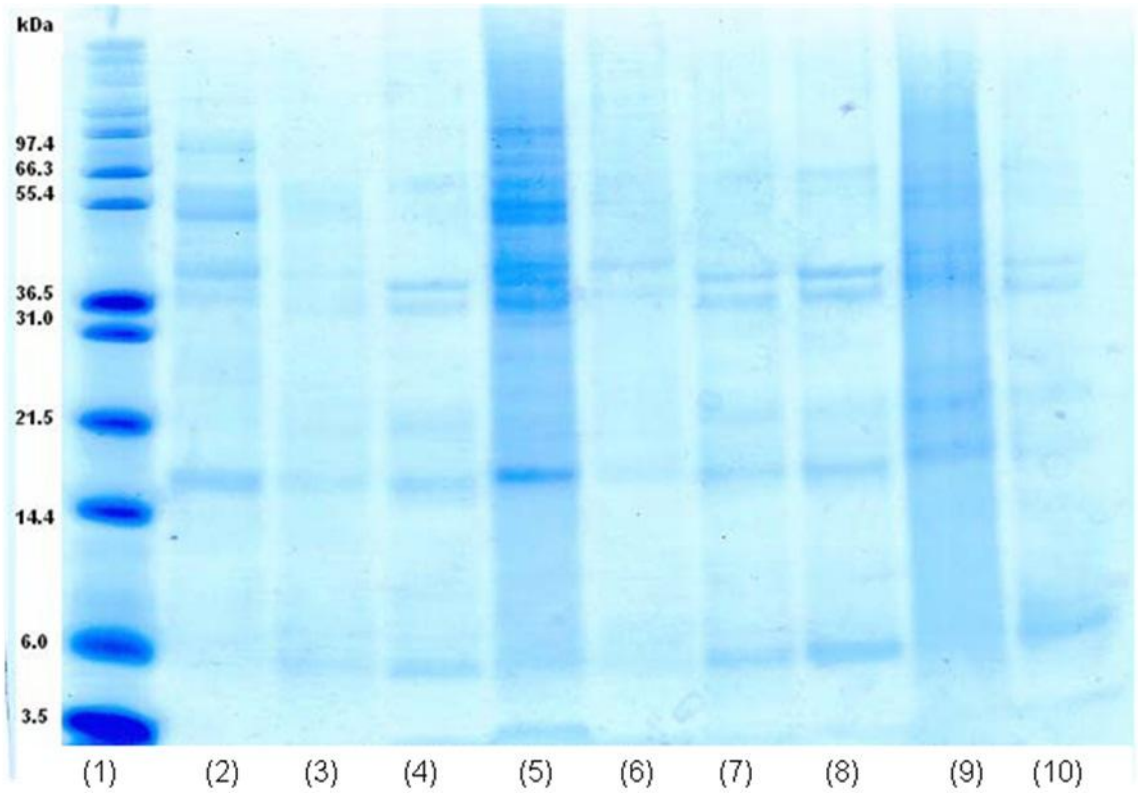

Figure 4.18 SDS-PAGE analysis of protein fraction of dehydrated carrots subjected to different blanching treatments. (1) Markers of molecular weight, (2) FD (control), (3) D-C60-40-M, (4) D-CB-1-M, (5) D-C95-5-M, (6) D-USP60-10-M, (7) D-CS-2-S, (8) D-CB-1-S, (9) D-C95-5-S, (10) D-USP70-15-S.

Physical changes during drying of carrot samples

Although rehydration cannot be considered as a reversible process to dehydration, since blanching and drying can provoke tissue disruption that gives rise to a certain hysteresis during rehydration (Lewicki, 1998a), this property is highly correlated with consumers' acceptance of dried products.

Carrot samples processed in this study were evaluated for their rehydration ability after drying and the results are shown in **Figure 4.19**. The RR values ranged from 4.2 to 14.8. Carrots blanched with steam and boiling water presented RR values close to 5, significantly lower than that of the FD sample. Giri & Prasad (2009) also found higher RR values in freeze-dried mushrooms (4.3) than in the same type of vegetable dried by convection (2.5); however, in both cases no pre-treatment was previously applied. Soria et al. (2010) reported RR values within the range of 5.7-7.2 for commercially dehydrated carrots and 6.7 for laboratory freeze-dried samples previously blanched by boiling water for 1 min. Similar values were obtained by Gamboa-Santos et al. (2012c) in carrot samples industrially processed by hot-air after a previous blanching at 98 °C for 20 min. In this study, the highest RR values were found in dried samples blanched at 95 °C for 5 min and at 60 °C for 40 min, in agreement with their highest initial content of moisture, as shown in **Figure 4.17**.

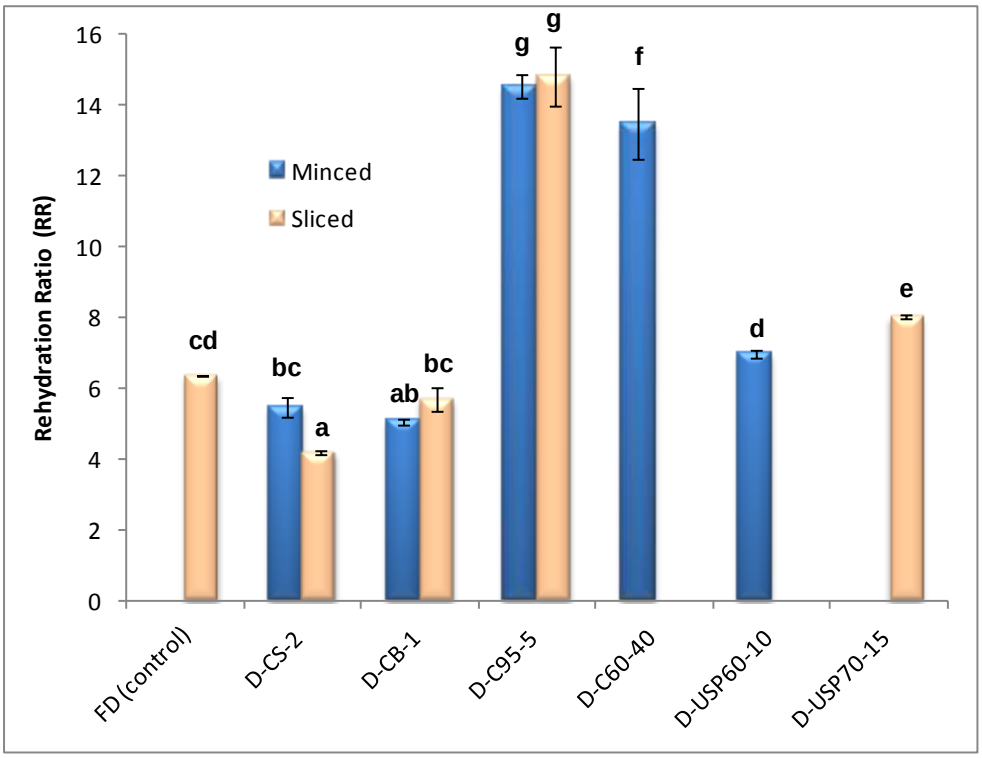

Figure 4.19 Rehydration ratio (RR) of carrot samples under analysis (Table 4.25). Mean of 3 replicates and standard deviation in error bars. Samples with the same letter (a-g) showed no statistically significant differences for their mean values at the 95% confidence level.

The RR of dried samples blanched by US, particularly that of the D-USP70-15-S sample, were significantly ($p<0.05$) higher than those of D-CS-2 and D-CB-1 carrots. In a study on accelerated drying of mushrooms, Brussels sprouts and cauliflower by power US, Jambrak et al. (2007a) found intermediate rehydration properties of dried samples (60 °C, 0.3 m/s) previously treated by US with a probe (20 kHz) or bath (40 kHz), as compared to freeze-dried samples and dried samples previously blanched at 80 °C for 3 min.

Some authors have postulated that when PME activity is present, cell walls become harder, avoiding ulterior thermal damage and this could imply a decrease in the rehydration level (Heredia-León et al., 2004). However, when considering samples processed in this paper, carrots D-C60-40-M, D-USP60-10-M and D-USP70-15-S presented high values of RR and, coincidently, these carrot samples showed a certain residual activity of PME after blanching (Gamboa-Santos et al., 2012a). On the contrary, samples blanched with steam, boiling water and water at 95 °C did not present any PME residual

activity and their RR after drying was highly variable, as indicated in **Figure 4.19**. Therefore, within the range of experimental conditions studied here, there was no apparent correlation between PME activity and the rehydration properties. These results could probably be due to the fact that the residual PME activity of samples D-C60-40-M, D-USP60-10-M and D-USP70-15-S after blanching could have disappeared during the drying process. Therefore, other effects such as the physical changes on microstructure could be the main factor affecting RR.

The microstructure analysed by SEM of the FD and convective dried samples after blanching by conventional and US treatments is shown in **Figure 4.20**. As can be observed, FD carrots show a perfect organization of the vegetal tissue. Cells are polyhedrical, similar sized and uniformly distributed through the matrix. This is due to the fact that during water sublimation in freeze drying, hardly any change is produced and this contributes to a great extent to preserve the original organization of the cellular parenchyma. However, in the case of samples thermally processed, the cell walls are more or less twisted, depending on the severity of the treatment. Particularly under the most severe conditions (95 °C, 5 min), the original cellular structure is noticeably transformed and a structural collapse is provoked, probably due to the degradation of pectinacious material during processing and the appearance of intercellular voids. According to microstructural observations, Sanjuán et al. (2005) indicated that conventional blanching of carrots at 95 °C for 1 min tends to cause separation along their cell walls, forming voids among the phloem parenchyma cells. These voids would be filled with water during rehydration, thus showing the slightly higher rehydration properties. Similar results were reported for carrot samples treated at 105 °C for 10 min, steamed-blanched carrots and slightly cooked carrots (Fuchigami et al., 1995; Kidmose & Martens, 1999; Rastogi et al., 2008). Thus, the highest RR found in D-C95-5-M, D-C95-5-S and D-C60-40-M carrot samples could be due to their loss of structure, which can facilitate water diffusion during rehydration.

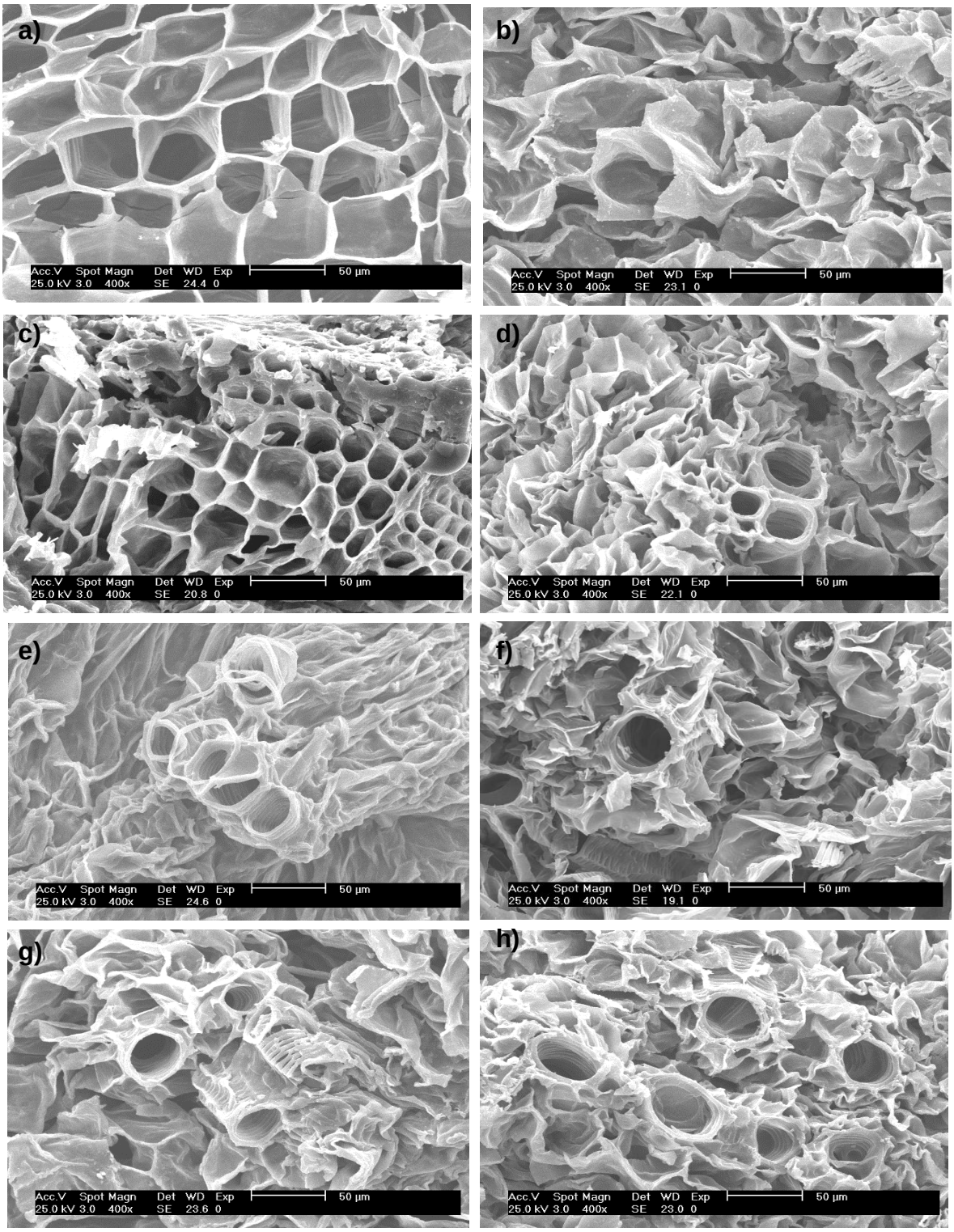

Figure 4.20 Electron microphotographs of dried carrots (400 x). a: FD (control); b: D-CS-2-M; c: D-CB-1-M; d: D-CB-1-S; e: D-C95-5-S; f: D-C60-40-M; g: D-USP70-15-S; h: D-USP60-10-M.

Samples blanched by US (D-USP70-15 and D-USP60-10) also presented a noticeably modified cellular structure; however, in this case, the mechanism involved is related to the creation of a porous material (Cárcel et al., 2012). According to Fernandes et al. (2011), the cavitation and micro-streaming provoked by US can contribute to the formation of microscopic channels in the vegetal tissues. All of this could justify the high RR values found for these carrot samples (**Figure 4.19**). García-Noguera et al. (2010) reported a breakdown of tissue structure in strawberries pre-treated in an ultrasonic bath at 30 ºC for 60 min, whereas other authors did not find important differences in Malay apples processed under similar conditions (Oliveira et al., 2011).

Conclusions

It can be concluded that the drying process and quality parameters of convectively dehydrated carrots are highly dependent on the blanching type and conditions. Samples conventionally processed under long time-low temperature (LTLT) conditions (60 °C, 40 min) or under the most severe conditions (95 °C, 5 min) were dehydrated faster and showed the highest rehydration ratio and loss of carbohydrates. The highest advance of the Maillard reaction was observed in carrot samples subjected to blanching at 95 °C for 5 min, as evidenced by its 2-FM-AA content and by the changes in its protein pattern determined by SDS-PAGE. However, samples conventionally blanched with boiling water or by steam presented a lower rate of drying and lower losses of carbohydrates and formation of 2-FM-AA. Samples processed by US showed an intermediate dehydration rate and TPC levels and rehydration properties similar to those of the control sample. To the best of our knowledge, this is the first time that the effect of blanching on the subsequent evolution of MR during drying of vegetables has been assayed. The results obtained in the present paper underline the usefulness of furosine as a marker of carrot processing, particularly if avoiding losses of nutritive value due to the participation of lysine in the MR is intended.

4.2.1.2.2 Contenido de vitamina C y propiedades sensoriales en zanahorias deshidratadas previamente escaldadas por ultrasonidos y convencionalmente

Vitamin C content and sensorial properties of dehydrated carrots blanched conventionally or by US

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Food Chemistry, 136 (2013), 782-788, doi: 10.1016/j.foodchem.2012.07.122

Abstract

Vitamin C content and sensorial properties have been evaluated in air-dried carrots previously subjected to different US (US) or conventional blanching pre-treatments. In addition, mass spectral fingerprints obtained by the Headspace ChemSensor System have been evaluated for the first time for classification of carrots according to their processing. Conventional blanching treatments at high temperature gave rise to carrots with retention of vitamin C in the range 37.5-85%, whereas carrots blanched conventionally at 60 °C and by US-probe at temperatures up to 60 and 70 °C showed vitamin C retention values lower than 4%. Regarding sensorial analysis of rehydrated carrots, US pre-treated samples presented acceptable quality, and no statistically significant differences with respect to conventionally blanched carrots, were detected. In spite of this, differentiation of samples processed under comparable intensity conditions and/or with similar composition was possible from their mass spectral fingerprints after chemometric data analysis.

Introduction

Carrot (*Daucus carota* L.) is considered one of the vegetables whose consumption, both fresh and processed, has increased over the past years due not only to the nutritional and health benefits this vegetable provides, but also to the introduction of new carrot-derived products (Alasalvar et al.,

2001). In addition, its pleasant flavour is one of the main reasons for its acceptance by consumers; volatiles (mainly terpenes and sesquiterpenes) and sugars being the main compounds that account for the distinctively carrot-like flavour (Alasalvar et al., 2001).

Among the different processes that can be applied to fresh carrot to obtain a product with longer shelf life and/or new characteristics, dehydration by hot-air is probably the most popular (Prakash et al., 2004). While the shelf-life might be increased up to one year after dehydration, the quality of dehydrated vegetables might also be negatively affected as compared to that of fresh foodstuff (Negi & Roy, 2001).

Apart from drying conditions (temperature, air-rate, etc.), other previous and subsequent sample treatments might affect the quality of the final product (Negi & Roy, 2001). In this respect, blanching is one of the most commonly used pre-treatments to inactivate the enzymes responsible for quality deterioration of processed carrots and it can be carried out under high temperature (Shivhare et al., 2009) or low temperature (Mohamed & Hussein, 1994) conditions. However, similarly to other thermal processes, blanching and drying have been described to affect several nutritive and bioactive compounds of vegetables including, among others, their vitamin C content (Drake et al., 1981). Moreover, both processes can also modify the quality of carrot flavour as a consequence of changes in volatile and sugar profile after these operations (Shamaila et al., 1996; Soria et al., 2008a).

Application of alternative blanching methods and/or optimisation of drying conditions may result in processed carrots with better flavour, nutritive and bioactive characteristics. Ultrasound (US) blanching has recently emerged as a pre-treatment with positive results on the effective water diffusivity of fruits mainly processed by osmotic dehydration (Fernandes et al., 2011). In the case of carrots, hardly any research has been carried out on the potential of US as an alternative to conventional blanching. Rawson et al. (2011) reported higher retention of carotenoids and polyacetylenes in dried carrots subjected to a pre-treatment with a US probe (10 min under pulsed mode) than in dried carrots blanched at 80 °C for 3 min. More recently, Gamboa-Santos et al. (2012a) have compared the effect of different conventional blanching methods (water and steam water) and US pre-treatments on enzyme inactivation and leaching losses in carrots. Samples

blanched for 10 min by US-probe with generation of heat (temperature up to 60 ºC) showed similar characteristics to those conventionally treated at 60 ºC for a longer time (40 min).

Carrot aroma is generated by a number of volatile compounds of different functionality usually present at very low concentrations; relative volatile composition being dependent on carrot variety, harvesting conditions, pre-processing, processing and storage conditions, etc (Soria et al., 2008). Carrot volatiles have also been described to be highly correlated with different sensory attributes such as odour, taste and aftertaste and with consumer liking (Varming et al., 2004).

Although the common approach for carrot classification according to its volatile composition usually consists of the application of different fractionation techniques for isolation/preconcentration of volatiles previous to their GC-MS analysis (Shamaila et al., 1996; Soria et al., 2008c), new methodologies based on direct sampling-mass spectrometry (DS-MS) have been recently reported for this purpose (Marsili, 2011; Peña et al., 2002). In DS-MS, headspace volatiles sampled directly into the mass spectrometer give rise to a characteristic chemical fingerprint (mass spectrum corresponding to the global volatile profile) for every sample. The use of chemometrics for interpretation of patterns from these multivariate data allows fast and precise classification of samples according to different criteria. Thus, DS-MS has been used for classification of different olive oil classes (Peña et al., 2002) and of wines (Dirinck et al., 2006), among others. Regarding carrots, this methodology has only been applied for discrimination of diseases of stored carrots (Vikram et al., 2006), and no study has yet addressed the application of DS-MS for classification of carrots according to their processing. Advantages of this methodology include minimal sample preparation and high throughput as no prior chromatographic separation is required.

In view of the studies cited above, the main objectives of the present work were: (i) the evaluation in hot-air dried carrots of changes in vitamin C retention and in sensorial properties associated with a previous treatment by US or conventional blanching; (ii) the study of mass spectral fingerprints obtained by the Headspace ChemSensor System (MS-electronic nose) for classification of carrot samples according to their processing.

Materials and methods

Sample preparation

Fresh carrots (*Daucus carota* L. var. Nantesa) were taken from a single batch that was purchased at one time in a local market in Madrid (Spain). Carrots were stored at 4°C for less than a week until processing. Carrots were properly washed in tap water to remove external impurities. Then, samples were cut into slices of 24 mm diameter and 4 mm thickness and as minced carrots (1-2 mm).

Blanching

Table 4.28 summarizes all conventional and US blanching treatments carried out on carrot samples. Processing conditions and sample geometry were chosen on the basis of optimal results in terms of enzyme inactivation and leaching losses previously reported by Gamboa-Santos et al. (2012a).

Table 4.28 Processing conditions used during conventional / US blanching and further convective drying of carrots

Sample code	Carrot geometry	Blanching conditions	Drying time(h)[a]
CS-2	Sliced	Steam (98 ºC, 2 min)	0
CB-1	Sliced	Boiling water (98 ºC, 1 min)	0
C95-5	Sliced	Hot water (95 ºC, 5 min)	0
C60-40	Minced	Hot water (60 ºC, 40 min)	0
USP60-10	Minced	US probe (up to 60 ºC, 10 min)	0
USP70-15	Sliced	US probe (up to 70 ºC, 15 min)	0
D-CS-2	Sliced	Steam (98 ºC, 2 min)	9
D-CB-1	Sliced	Boiling water (98 ºC, 1 min)	9
D-C95-5	Sliced	Hot water (95 ºC, 5 min)	9
D-C60-40	Minced	Hot water (60 ºC, 40 min)	7
D-USP60-10	Minced	US probe (up to 60 ºC, 10 min)	7
D-USP70-15	Sliced	US probe (up to 70 ºC, 15 min)	9

[a]Dried samples were processed at 46ºC and at a air drying rate of 4.9 ms^{-1}.

Ultrasound treatments

For US treatments, samples of 40 g were placed into 250-mL Erlenmeyer flasks filled with 200 mL of distilled water and were sonicated using an ultrasonic system (450 Digital Sonifier, Branson Ultrasonics Coorporation, Danbury, CT, USA). This sonicator was equipped with a

temperature sensor and a tip of 13 mm diameter directly attached to a disruptor horn (20 kHz, 400 W full power) which was immersed 2 cm in the liquid. Experiments were carried out with generation of heat: US blanching for 10 min at temperatures up to 60 °C (USP60-10), and for 15 min at temperatures up to 70 °C (USP70-15). The ultrasonic density, calculated according to Jambrak et al. (2007b), was 0.26 W/cm^3.

Conventional treatments

Using the same carrot - distilled water ratio as above mentioned, carrot samples were subjected to blanching with boiling water for 1 min (CB-1), with water at 95 °C for 5 min (C95-5) and at 60°C for 40 min (C60-40) using a hot-plate with temperature control (IKA RCT Basic Labortechnik, Staufen, Germany). Carrot sample CS-2 was pre-treated by steam blanching for 2 min using an autoclave (CERTOCLAV CV-EL GS, Austria) operating under atmospheric pressure conditions.

All assays (US and conventional) were performed in duplicate. After treatments, samples were cooled in an ice-water bath and conveniently drained and dried with absorbent paper to remove the excess of distilled water.

Drying procedure

In order to evaluate the contribution of air-drying to changes in bioactivity and sensory properties of carrots, additional experiments by convective drying were carried out on samples previously subjected to either conventional or US blanching (**Table 4.28**).

Blanched carrot samples (80 g) were dried using a computer controlled (Edibon Scada Control and Data Acquisition Software) air tray-dryer (SBANC, Edibon Technical Teaching Units, Spain). This system consists of three main sections: (i) fan unit with control of the air-flow rate, (ii) control of temperature (seven thermo-hygrometers, ST1-ST7) and (iii) drying compartment (load cell with four drying trays). ST7 was chosen as the process temperature since it was the wet bulb closest to the samples. ST7 registered a temperature of 46 ºC by setting the resistance thermometer at a

temperature of 60 ºC. The air-flow was parallel to the samples and the air-rate was selected with the AVE sensor at 4.9 m/s. During the drying process, the weight of the samples was automatically monitorized by using the load cell of the system. Minced carrots were dried for 7 h and sliced carrots for 9 h.

The dry matter (DM) content of carrots was gravimetrically determined by drying the samples in a conventional oven at 102 ºC until constant weight (AOAC method, 1990a). After drying, all samples presented DM contents within the range 85-89%.

Determination of vitamin C

The procedure employed to determine total vitamin C (ascorbic acid plus dehydroascorbic acid) was the reduction of dehydroascorbic acid to ascorbic acid, using D,L-dithiothreitol as reducing reagent (Plaza et al., 2006). Carrot extracts were prepared by adding 12.5 mL of 0.4% oxalic acid to 0.25 g of carrot samples and homogenizing for 1 min at 13,500 rpm using an Ultra-Turrax T-25 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany). Oxalic acid was used to inhibit further degradation of vitamin C (Erle, 2001). After addition of 2.5 mL of a 5 mg/mL solution of D,L-dithiothreitol, carrot extracts were kept at room temperature in the darkness for 30 min. Once the volume of the slurries was made up to 25 mL with Milli-Q water, they were centrifuged at 3,200*g* for 5 min. The supernatant was filtered through 0.45 µm syringe filters. Carrot extracts were made in duplicate.

Total vitamin C content of carrots was determined by liquid chromatography with diode array detector (RP-HPLC-DAD) on an Agilent Technologies 1220 Infinity LC System – 1260 DAD (Boeblingen, Germany). The separation of vitamin C was carried out with an ACE 5 C_{18} column (ACE®, UK) (250 mm, 4.6 mm i.d., 5 µm) thermostated at 25 °C, using 5 mM KH_2PO_4 at pH 3.0 as the mobile phase. Elution was done under isocratic conditions at a flow rate of 1 mL/min for 10 min. Injection volume was 20 µL and data acquisition and processing were performed using the Agilent ChemStation software (Agilent Technologies, Germany).

Quantitation was performed by the external standard method, using a commercial standard of ascorbic acid (Sigma) in the range 0.3-50 mg/L. Determination coefficient obtained from this calibration curve, which was linear over the range studied, was R^2 = 0.999. Chromatographic repeatability (n=5) and method repeatability (n=2) were estimated and the relative standard deviation (RSD) calculated was below 3%. Results were expressed as milligrams of total vitamin C per 100 g DM.

Data were subjected to one-way analysis of variance (Fisher's least significance difference (LSD) test) by applying the Statgraphics 4.0 program (Statistical Graphics Corporation, Rockville, Md) for Windows.

Sensory evaluation

After drying, carrot samples were rehydrated in boiling water for 10 min using a sample:water ratio of 1:30 (Prakash et al., 2004). The sensory analyses of these samples were carried out by a taste panel of 14 semi-trained judges who were familiarized with samples, attributes and their definitions during two orientation sessions.

A triangle test procedure (ISO Standard 4120) was followed. Panellists were presented with 2 groups of 3 samples each, distributed so that in each group 2 samples were the same and another was different in a randomized order. Panellists were asked to identify the odd sample, paying special attention to the taste and texture. Rehydrated carrot samples were also evaluated in a hedonic test. Two different sessions were performed: D-C60-40 and D-USP60-10 carrot samples were coded and presented at random order to the panellists in the first session and D-CS-2, D-CB-1, D-C95-5 and D-USP70-15 carrot samples in the second. The panellists were asked to indicate their preference for each sample, mainly based on texture and taste. A balanced 8-point hedonic rating was employed for all the attributes evaluated, where 1 denoted "like very much" and 8 indicated "dislike very much" (Sancho, Bota & de Castro, 2002).

Data were subjected to one-way analysis of variance (Fisher's least significance difference (LSD) test) by applying the Statgraphics 4.0 program for Windows.

Discrimination analysis using the ChemSensor System (MS e-nose)

Dehydrated samples were ground and aliquots of 0.3 g of each treatment plus 300 µL of water were placed in a 10 mL vial and were hand-crimper sealed with an inert septum. DS-MS analyses were performed with a ChemSensor 4440B system (Agilent Technologies, Palo Alto, CA, USA), which can be considered as a MS electronic nose. It comprises of two modules. The first one is a 44-vial autosampler for headspace sampling (Agilent HS 7694) that includes an oven to heat the samples and to form the headspace (120 ºC for 30 min), and a six-port injection valve with a 3-mL loop. Helium at 16 psi for 18 s was used for pressurizing the vial. The second module is a quadrupole mass spectrometer detector (MS5973N), operated in full scan mode (*m/z* 50-200) at 1.43 scans/s. The ionization energy was 70 eV. The transfer line, source, and quadrupole temperatures were set at 140, 230, and 150°C, respectively. Data were acquired and a chemometric analysis was performed using Pirouette data analysis software (v3.11, Infometrix Inc., Bothell, WA).

Chemometric procedures. Matrix of *m/z* abundances was obtained from mass spectral fingerprints collected for every sample and subjected to statistical analysis. First, an unsupervised technique such as Principal Component Analysis (PCA) was applied in order to reduce the dimensionality of the data matrix and to find internal structures or clustering of data. Later, a supervised technique, Soft Independent Modeling Class Analogy (SIMCA), was used to obtain adequate classification procedures at the 95% confidence level. SIMCA develops principal component models to classify samples into discrete categories. It is based on the concept of proximity, the assumption that if a set of measurements for an unknown sample is very similar to that of a specific group, then the unknown is likely to be a member of that group. Although the ultimate goal of SIMCA is the reliable classification of new samples (i.e., unknowns), in this study SIMCA was used for discrimination of samples according to their processing. Thus, samples were visually classified by observing its position in the Coomans plot where the multiple thresholds divide the plot space into subregions of membership and not: a) the sample fits only one pre-defined category (a sample in the NW quadrant is a member only of the *x* axis class, and a sample falling in the SE quadrant is a member

only of the *y* axis class); b) the sample does not fit any pre-defined categories (sample in the NE quadrant); c) the sample fits into more than one pre-defined category (sample in the SW quadrant) (Coomans et al., 1984).

Results and discussion

Effect of processing conditions on vitamin C

The retention of vitamin C is often used as an estimation of the overall nutritional quality of food products, particularly, vegetables (Goula & Adamopoulos, 2006). The losses of this vitamin are mainly attributed to its solubility in water and to its sensitivity to high temperatures and oxidation conditions (oxygen, pH and metal ions) (Davey et al., 2000). **Table 4.29** shows the content of vitamin C (mg/100 g DM) and the corresponding percentages of vitamin retention of the different carrot samples (dried and/or blanched) analysed in the present study. The content of vitamin C of raw carrot was close to data previously reported by other authors (Negi & Roy, 2001; Frias et al., 2010).

Considering the effect of blanching, as can be seen in **Table 4.29**, the highest content of vitamin C, representing 85% retention of this vitamin, was found in CB-1 carrot sample. Frías et al. (2010) reported approximately the same retention level (80%) of vitamin C in samples of carrots subjected to the same blanching conditions. After CS-2 treatment, the percentage of retention was also very high (81%), and no significant differences ($p>0.05$) were found with respect to previous treatment. Drake et al. (1981) studied the influence of blanching method on the quality of selected vegetables and they found that water and steam blanched asparagus and green beans showed similar ascorbic acid concentration. Lin & Brewer (2005) observed in peas that steam blanching resulted in significantly better ascorbic acid retention than treatments with boiling water for equal blanching time. In the case of blanching treatments carried out at 95 ºC for 5 min (C95-5) (**Table 4.29**), carrots presented a considerable reduction (62.5%) in the content of vitamin C. However, Shivhare et al. (2009), among the different assayed conditions, proposed this combination of temperature and time together with

0.05 N acetic acid solution, as the best blanching treatment of carrots destined to juice elaboration. Lin et al. (1998) reported that blanching of carrots at 90 ºC for 7 min before drying can preserve 57.5% of vitamin C content and Negi and Roy (2001) found 87.6% of retention of this vitamin after blanching of carrots at 95 ºC for 90 s. In agreement with our experimental data, all these results highlight the great influence of small changes in blanching conditions (temperature, time, sample geometry, blanching water:carrot weight ratio, etc) on preservation of vitamin C content of carrots.

Table 4.29 Effect of conventional/US blanching and further convective drying on vitamin C content (mean ± SD) of carrots under study

Carrot samples	Vitamin C	
	Content (mg/100 g DM)	Retention (%)
Raw	35.57 ± 4.20[a]*	100
CS-2	28.88 ± 0.10[d]	81.2
CB-1	30.24 ± 0.82[d]	85.0
C95-5	13.33 ± 1.35[c]	37.5
C60-40	0.48 ± 0.01[b]	1.3
USP60-10	0.25 ± 0.02[b]	0.7
USP70-15	1.31 ± 0.18[b]	3.7
D-CS-2	14.30 ± 0.22[c]	40.2
D-CB-1	18.77 ± 1.40[f]	52.8
D-C95-5	7.32 ± 0.17[e]	20.6
D-C60-40	tr**	-
D-USP60-10	tr	-
D-USP70-15	1.05 ± 0.27[b]	2.9

*Samples with the same superscript showed no statistically significant differences for their mean values at the 95.0% confidence level.
**tr: traces

Regarding low temperature long time (LTLT) conventional blanching treatments, as shown in **Table 4.29**, C60-40 assay was the most drastic and resulted in the highest loss of vitamin C. These results could be explained by the noticeable leaching loss associated with long blanching times and/or the sample geometry, since C60-40 carrots were minced and presented higher specific surface than the slices used in the other conventional treatments (**Table 4.28**). In spite of the mild temperature used (60 ºC), no oxidation of ascorbic acid due to residual ascorbic acid oxidase was suspected since, according to Rayan et al. (2011), hardly any residual activity of this enzyme is presented under these experimental conditions.

In the case of carrot samples subjected to US blanching (**Table 4.29**), the loss of vitamin C was also very high, particularly in the case of USP60-10, which was close to 99%, as shown for C60-40 assay. These results are in agreement with the similar losses by leaching of total solids and soluble sugars and with the comparable results on inactivation of peroxidase and pectinmethyesterase previously reported for these samples (Gamboa-Santos et al., 2012a). When USP60-10 and USP70-15 samples were compared, no significant differences ($p>0.05$) were found for the retention of vitamin C.

The main mechanism involved in the loss of vitamin C during US blanching treatments might be the formation of microchanels during cavitation which facilitate the transport of food constituents, especially soluble nutrients (Mothibe et al., 2011). In agreement with this, Opalic et al. (2009) reported that prolonged US pre-treatment in samples with the same geometry led to a decrease in total phenols and flavonoids as well as in the antioxidant capacity of dried apples.

The effect of drying on the retention of vitamin C of the different blanched carrot samples studied was also evaluated (**Table 4.29**). Since all samples were dried under the same conditions (46°C; 4.9 m/s), the observed variations in the final content of this vitamin (traces to 18.77 mg/100 g DM), as we have indicated above, were due to the different blanching procedures. The highest retention was found in D-CS-2 and D-CB-1 carrot samples; however, D-C95-5 carrot lost most of its content of vitamin C by leaching during blanching and the losses during dehydration were similar to those observed in D-CS-2 and D-CB-1 samples. With respect to the other samples analysed (D-C60-40, D-USP60-10, D-USP70-15), very low amounts of vitamin C were detected after drying since the prior blanching treatments were very severe.

The destruction of thermolabile vitamin C during the drying process was mainly due to the effect of drying time, since the temperature of the process was rather mild (46 °C). In agreement with this, Mohamed & Hussein (1994) observed that ascorbic acid of carrot was easily damaged by long drying times, whereas carotenoids were more sensitive to drying temperature than to drying time. Negi & Roy (2001) reported 46% of vitamin C retention in carrots blanched at 95 °C for 30 s after drying at 65 °C, whereas Frias et al. (2010a) reported retention data of this vitamin within the range 43-50% in

carrot samples blanched in boiling water (60 s) and subsequently subjected to drying by convection at temperatures of 43-52 °C for 6 h.

Sensory evaluation

Sensory evaluation of the rehydrated carrot samples was carried out to obtain preliminary information on consumer's preference and product acceptance. Sensory assessment of dried samples was not performed, as Lin et al. (1998) found that colour, appearance, texture, aroma/flavour and overall acceptability of hot air-dried carrot slices were greatly improved when they were rehydrated and, moreover, dried carrots will mostly be consumed in rehydrated form.

In the triangle test, samples D-C60-40 and D-USP60-10 could not be distinguished in relation to the flavour and texture by the sensory panel since only 50% of panellists found the odd sample. The mean overall liking scores of the evaluated samples are shown in **Table 4.30** and, as it can be observed, no significant differences ($p>0.05$) were found between the analysed samples. The score values were within the range 3.7 (close to "like slightly") for D-C60-40 and 3.2 (close to "like moderately") for D-USP60-10 carrot samples. When D-CS-2, D-CB-1, D-C95-5 and D-USP70-15 rehydrated carrot samples were compared in the hedonic scale (**Table 4.30**), the liking scores were similar among them and within the rating range "like moderately"- "like slightly", previously mentioned for the remaining samples here evaluated.

Table 4.30 Overall scores (mean ± SD) of rehydrated carrots subjected to different conventional/US blanching treatments prior to drying

Carrot samples	Score*
D-C60-40	3.7 ± 0.9
D-USP60-10	3.2 ± 1.0
D-CS-2	3.0 ± 1.2
D-CB-1	3.6 ± 1.0
D-C95-5	3.5 ± 1.3
D-USP70-15	3.5 ± 1.2

*Samples showed no statistically significant differences for their mean values at the 95.0 % confidence level.

The obtained scores could be considered low for a highly appreciated product like carrots, but this fact could be explained considering the unavoidable losses of carbohydrates taking place mainly during blanching. Moreover, changes in volatile composition by evaporation, degradation, leaching and/or formation of new compounds through blanching and processing could also support these results. In agreement with this, Shamaila et al. (1996) reported that blanching exerts a significant negative effect on the sensory attributes of carrots and their overall impression.

As it is well-known, carbohydrates together with volatiles are mostly responsible for the pleasant flavour and consumer acceptance of carrots (Alasalvar et al., 2001). Although no significant differences ($p>0.05$) were found, it is remarkable that the sample with the best score (3.0) was that corresponding to steam blanching (D-CS-2), probably due to the fact that this procedure has a lower impact on the losses of carbohydrates and volatiles than the conventional ones (Shamaila et al., 1996; Wang et al., 1997; Gamboa-Santos et al., 2012a).

In general, the panellists highlighted the difficulty of the test since the assayed samples presented similar attributes and overall quality. Since drying and rehydration were the same in all cases, it seems that differences caused by blanching were minimized during the subsequent steps of processing. Similarly, Lin et al. (1998) found no significant differences in overall acceptability of rehydrated carrots previously blanched (at 90 ºC during 7 min) and processed by air drying, vacuum microwave drying, and freeze-drying; however, differences were found when the non-rehydrated samples were compared.

Samples subjected to US blanching prior to drying by convection presented an acceptable quality, similar to that of carrots blanched by different conventional methods. Opalic et al. (2009), in a study on the use of an ultrasonic bath for 9-54 min for blanching of apples before drying, found a decrease in the sensory characteristics with the time of processing. When US were applied to osmotic drying of fruits, consumers preferred these samples because of their high sugar content (Mothibe et al., 2011).

Discrimination analysis using the ChemSensor System (MS e-nose)

Mass fingerprints obtained by using the ChemSensor methodology for samples under study (**Table 4.28**) were subjected to PCA in order to explore their unsupervised grouping. As shown in **Figure 4.21** (PC1 vs PC2, 87.33% of variance explained), precision of the method was good for all the samples analysed, with scores corresponding to the same treatment being plotted close to each other. Considering the different blanching types assayed, only C60-40 pre-treatment and its corresponding dehydrated carrots (D-C60-40) were plotted apart based on their high PC1 scores (> 50). On the other hand, from the similar location in this figure of blanched and their subsequently dehydrated carrots, it can be highlighted the higher impact of blanching conditions over identical dehydration on volatile composition of carrots, particularly for those samples processed under the most energetic conditions.

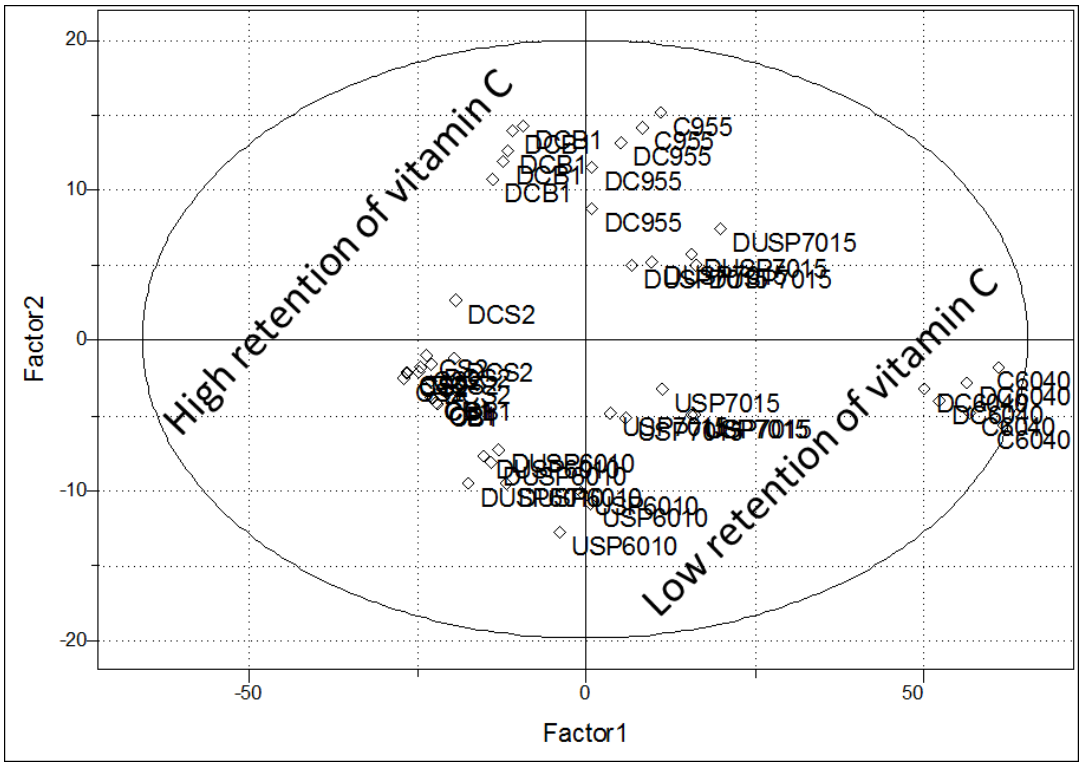

Figure 4.21 Principal component biplot of mass spectral fingerprints corresponding to carrot samples under analysis.

The scores plot of samples in **Figure 4.21** also shows the coincidence of this classification with their vitamin C content. Thus, samples plotted at the right-bottom of this figure showed very low retention of vitamin C (C60-40,

D-C60-40, USP70-15, D-USP70-15, USP60-10, D-USP60-10), whereas samples plotted at the left-top showed high retention of this vitamin (CB-1, CS-2, D-CB-1, D-CS-2, C95-5, DC95-5).

Based on ChemSensor testing using *m/z* intensity results, samples were visually classified by observing its position in Coomans plots. As example, **Figure 4.22** shows some of them. Samples for every category clustered nicely, being this a prior requirement for its classification.

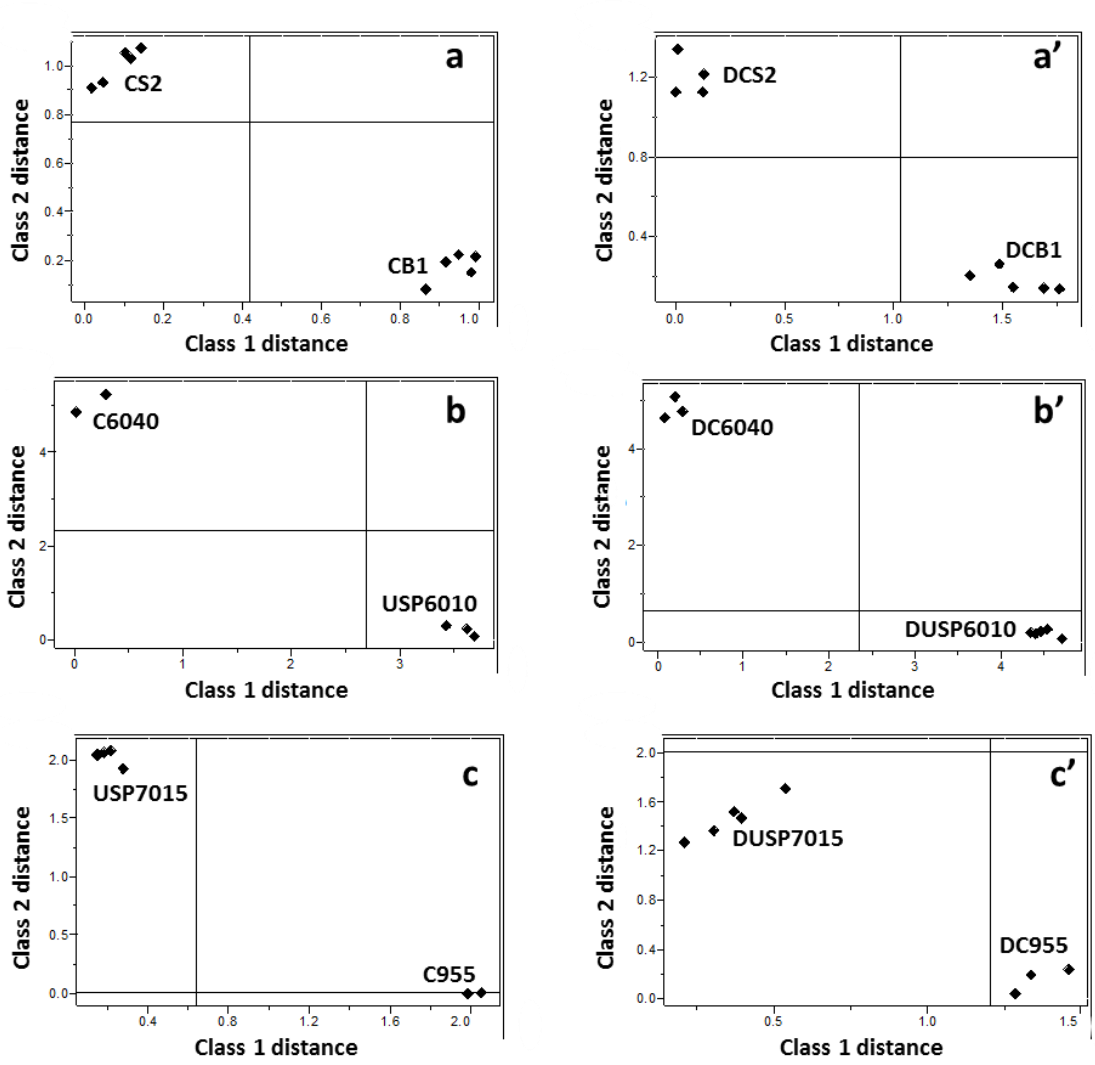

Figure 4.22 Coomans plots. For identification of samples, see Table 4.28**.**

Regarding the effect of blanching in samples not further dried, the pairs of samples CS-2 vs CB-1 (**Figure 4.22a**), C60-40 vs USP60-10 (**Figure 4.22b**), USP70-15 vs CS-2 and USP70-15 vs CB-1 were clearly classified in the correct class. It is noteworthy that samples subjected to similar blanching

(CS-2 and CB-1) were properly separated in the Coomans plot. Similarly, different treatments (C60-40 and USP60-10) which gave rise to the same chemical changes during the leaching of components (Gamboa-Santos et al., 2012a) were also correctly classified. Only some comparisons such as the pair USP70-15 vs C95-5 (**Figure 4.22c**) did not show an evident separation. Thus, whereas the USP70-15 samples were properly classified, the C95-5 samples were not, as they were borderline cases lying close to one of the thresholds.

When comparing carrots subjected to different blanching treatments and further dried under identical conditions, results were similar to those of non-dehydrated samples: D-CS-2 vs D-CB-1 (**Figure 4.22a'**), D-C60-40 vs D-USP60-10 (**Figure 4.22b'**), D-USP70-15 vs D-CS-2 and D-USP70-15 vs D-CB-1 were correctly classified, whereas samples D-USP70-15 vs D-C95-5 (**Figure 4.22c'**) were not properly identified as members of its actual categories. It can be concluded from these results that blanching conditions were the predominant factor affecting the global volatile composition of carrots here analysed, all of them dehydrated under identical experimental conditions. Furthermore, Chemsensor results allowed the differentiation of samples indistinguishable for 50% of the members of the taste panel (D-C60-40 vs D-USP60-10).

Conclusions

The high solubility in water of ascorbic acid makes the inevitable losses by leaching, associated to any of the blanching treatments assayed, responsible for the reduction to a certain extent of the content of this important vitamin. Taking into account the content of vitamin C, the samples with the highest retention were those subjected to conventional blanching at high temperature and short times. With respect to samples subjected to US blanching prior to drying by convection, the most striking feature was their acceptable organoleptic quality, similar to that of carrots blanched by different conventional methods. The statistical analysis of mass spectral fingerprints gathered by the ChemSensor methodology allowed the differentiation of samples with a similar composition and/or blanching

treatments, and indistinguishable for a taste panel of semi-trained judges. These results underline the usefulness of ChemSensor as a tool to classify processed carrot samples.

4.3. Procesos de deshidratación convectiva de zanahoria y fresa asistidos por ultrasonidos de potencia

4.3.1. Prefacio

Como se ha indicado en la Introducción (Apartado 1.3.2.1), la aplicación de US de potencia en la deshidratación ha emergido en los últimos años como una posible alternativa a los procesos convencionales por combinarse con temperaturas más suaves y reducir los tiempos de tratamiento. Además, se trata de una tecnología respetuosa con el medio ambiente. Aunque las posibilidades de deshidratar con US se conocen desde hace más de cinco décadas su desarrollo y aplicación ha sido muy lento debido a problemas en el diseño de generadores de alto rendimiento. Hasta la realización de la presente Memoria, la mayor parte de los trabajos estaban enfocados a la mejora de los sistemas ultrasónicos y al estudio de las cinéticas de pérdida de humedad. Así, como objetivo final de esta Memoria, se planteó estudiar en profundidad los principales cambios que podrían afectar a la calidad de zanahorias y fresas cuyo proceso de deshidratación estaba asistido por US. Si bien, en el caso de zanahoria existía algún precedente, en fresa no se había llevado a cabo ningún estudio. Para ello, se estudiaron los indicadores químicos y físicos seleccionados en apartados anteriores, por ser de gran utilidad como parámetros de calidad.

Inicialmente, se realizó un estudio (sección 4.3.1.1. *Chemical and physico-chemical quality parameters in carrot dehydrated by power ultrasound*) en un prototipo de deshidratación mediante US por contacto, desarrollado y patentado en el Instituto de Acústica del CSIC por el grupo del Dr. Gallego-Juárez (Gallego y col., 1996) que participaba en el proyecto dentro del cual se ha realizado el trabajo presentado en la esta Memoria. En dicho prototipo ya se había estudiado la cinética de pérdida de humedad en la deshidratación de zanahoria, pero no se conocía cuál era el efecto de este tratamiento sobre la calidad de dicho vegetal. Así, se realizaron ensayos con zanahoria (con o sin escaldado a ebullición) a temperaturas en el intervalo 20-60°C y tiempos de 75 a 120 min. A modo de comparación, se analizaron también muestras liofilizadas en el laboratorio y muestras comerciales. De todas las modificaciones estudiadas, lo más destacable fue el escaso avance

de la RM, ya que tan sólo se detectaron 2-FM-AA tras los tratamientos llevados a cabo a 60 °C durante 75 min. Las concentraciones halladas de 2-FM-AA fueron significativamente inferiores a las encontradas en muestras de zanahoria comerciales analizadas en este trabajo y en muestras industriales analizadas por otros autores. El resto de parámetros (carbohidratos, polifenoles totales, actividad antioxidante, perfil proteico) apenas sufrió cambios y fueron comparables a los obtenidos en muestras de zanahoria liofilizada.

Otra de las posibilidades de aplicación de US en la deshidratación es, durante el secado, utilizar sistemas sin contacto (Introducción, apartado 1.3.2.1.2.). Así, en el marco de una colaboración con el grupo de Análisis y Simulación de Procesos Agroalimentarios (ASPA) dirigido por el Dr. Mulet de la Universidad Politécnica de Valencia, se llevó a cabo un estudio sobre el secado convectivo de fresa asistido por US en un prototipo diseñado por dicho grupo. En este equipo ya se habían realizado numerosos trabajos sobre la modelización de la cinética de pérdida de humedad en varios vegetales y frutas, sin embargo, estudios sobre fresa aún no se habían abordado. En una primera fase (Apartado 4.3.1.2.1., *Effect of power ultrasound on the convective drying of strawberry*) se modelizó la cinética de secado considerando el modelo difusivo para lámina infinita, la resistencia externa a la transferencia de materia y el encogimiento, lo cual permitió determinar el efecto de los US de potencia en la eliminación del agua. Se realizaron ensayos a 40-70 °C y 0, 30 y 60 W de potencia a una velocidad constante del aire de secado (2 m/s), y se estudió la influencia de la temperatura y la energía acústica en el secado de fresa. En general, para todas las temperaturas ensayadas, se observó un efecto positivo sobre la velocidad de secado al aumentar la potencia de US, acortándose los tiempos de secado entre un 13 y 44%. Estos datos están en concordancia con lo previamente encontrado en la literatura para otros sustratos con diferente porosidad. Como parámetros cinéticos característicos del proceso se determinaron la difusividad efectiva (D_e) y el coeficiente externo de transferencia de materia (k) que fueron simultáneamente calculados ajustando el modelo mediante una función programada en Matlab. Los valores de *De* y de *k* se incrementaron para todas las temperaturas estudiadas a medida que aumentaba la potencia US aplicada, siendo menos importante el efecto al

aumentar dicha temperatura. Los resultados obtenidos en este trabajo pusieron de manifiesto la idoneidad de la aplicación de US en el secado convectivo de fresa. Al mismo tiempo, se remarcó la influencia positiva de los US sobre la cinética de pérdida de humedad, dado que, mediante este proceso se puede reducir la energía térmica requerida para llevar a cabo el secado de fresa.

Una vez realizado el estudio anterior, se evaluó el impacto de dicho tratamiento en la calidad del producto final (sección 4.3.1.2.2. *Impact of power ultrasound on the quality of convective dried strawberries*). Las condiciones de procesado fueron las indicadas en el trabajo anterior (40-70 °C; 0-60 W). A las temperaturas más suaves (40 y 50 °C), ningún tratamiento tuvo un efecto importante sobre la calidad del producto final, tan sólo se vio un ligero avance de las etapas iniciales de la RM. Sin embargo, bajo estas condiciones, no todos los ensayos condujeron a productos finales con la humedad requerida para ser microbiológicamente seguros.

A temperaturas más elevadas (60-70 ºC), se obtuvieron muestras de fresa con valores de humedad final adecuados, aunque se detectaron mayores modificaciones en los parámetros estudiados. Así, se observaron concentraciones significativamente más elevadas de los indicadores de la RM (2-FM-Lys+2-FM-Arg y 2-FM-GABA), especialmente a 70 °C, aunque no se observó efecto de los US sobre el contenido de estos indicadores en el producto final. Por lo que se refiere a la vitamina C, se observaron valores de retención elevados (≥65%), aunque dicha retención fue significativamente menor en los tratamientos con US. En general, teniendo en cuenta los niveles de 2-FM-AA y los valores de retención de vitamina C del producto final, se puede inferir que las fresas recién procesadas en el equipo de secado asistido por US presentaban una calidad superior a las comerciales. Las propiedades de rehidratación fueron muy similares a las encontradas en la bibliografía para este tipo de alimentos y se observó una disminución en la relación de rehidratación en las muestras de fresa deshidratada con y sin ultrasonidos a la temperatura más elevada (70 °C). Además, se evaluó la calidad microbiológica en las muestras tratadas sin US a 40 y 70 ºC y con US (60 W) a la misma temperatura, encontrándose en todos los casos, recuentos ≤ 3 log UFC/g. Una vez comprobada la viabilidad del sistema para obtener fresas con una adecuada calidad, se procedió a estudiar la estabilidad de las muestras

tratadas a 70 ºC con (60 W) y sin US, durante 6 meses de almacenamiento a 25 ºC, no encontrándose variaciones en los recuentos microbiológicos respecto al tiempo inicial. Estos resultados indicaron la seguridad del sistema de secado para obtener fresas deshidratadas estables a lo largo de su conservación. También se determinó que la pérdida de vitamina C en estas muestras tras los 6 meses de conservación fue próxima al 50%, obteniéndose un producto final con un contenido en dicha vitamina superior al de muestras comerciales de fresa deshidratada.

Los datos obtenidos en estos trabajos aportan suficiente evidencia científica para considerar los US como una tecnología eficiente en la deshidratación de zanahoria y fresa, no sólo por la reducción en los tiempos de procesado sino también por la calidad nutricional y microbiológica de los productos obtenidos.

4.3.1.1 Deshidratación por contacto. Parámetros de calidad químicos y físico-químicos en zanahorias deshidratadas por ultrasonidos de potencia

Chemical and physico-chemical quality parameters in carrots dehydrated by power ultrasound

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Journal of Agricultural and Food Chemistry (2010), 58, 7715-7722.

Abstract

The preservation of the quality and bioactivity of carrots dehydrated by power ultrasound (US) under different experimental conditions including prior blanching have been evaluated for the first time by measuring the evolution of the Maillard reaction and the changes in soluble sugars, proteins, total polyphenols, antioxidant activity and rehydration ability. This study also includes a comparison with a freeze-dried sample and data of commercial dehydrated carrots.

The synergic effect of US and temperature (60 ºC) increased the dehydration rate of carrots (90% moisture loss in only 75 min), while still providing carrots with a level of 2-FM-AA significantly lower than that of dehydrated commercial samples. Whereas a decrease in the content of reducing soluble sugars was observed with processing temperature, minor carbohydrates (*scyllo*- and *myo*-inositol and sedoheptulose) were rather stable irrespective of the US dehydration parameters. Blanching significantly improved the rehydration ability of US-dehydrated carrots, without increasing the loss of soluble sugars by leaching. As supported by the similarity of most quality indicators studied in both US-treated and freeze-dried carrots, the mild processing conditions employed in US dehydration gave rise to premium quality dehydrated carrots.

Introduction

Since fresh vegetables are highly perishable and difficult to preserve, the market of dehydrated vegetables has noticeably increased over the last years to provide consumers with long shelf-life food which are easy to handle and store (Lenart, 1996). Among the different dehydrated vegetables commercially available, carrots (*Daucus carota* L.) are increasingly being used in the elaboration of a number of food products (Ensminger et al., 1995).

Although different techniques have been reported in the literature for carrot dehydration, convective air drying is the process of choice for industrial applications. However, the operating conditions which are usually employed in convective drying (typically 40-80 ºC air temperature, 0.5-5 m s^{-1} air velocity and drying times as long as 20 h) (Doymaz, 2004b) may produce important chemical changes in the thermolabile carrot constituents (vitamins, phenolic compounds, etc) and in their physical properties (texture, rehydration ability, etc), resulting in a product of considerably lower quality when compared to the raw material (Hiranvarachat et al., 2008).

One of the most relevant chemical changes that occur at low water activity and high temperature conditions used for drying is the Maillard reaction (MR), that takes place between reducing carbohydrates and free amino groups of amino acids, peptides and proteins. 2-FM-AA obtained from the acid hydrolysis of the Amadori compounds formed at the early stages of the MR have been recently proposed as sensitive indicators for the early detection of changes in the nutritional value and organoleptic properties of several dehydrated vegetables (Cardelle-Cobas et al., 2005; Rufián-Henares et al., 2008); their contents being dependent on the vegetable species and their processing and/or storage conditions. It has also been suggested that these derivatives should be used in combination with hydroxymethylfurfural to assess the quality of hot-air dried carrots (Soria et al., 2009b).

A number of references have been reported on the content of major sugars in carrots of different varieties (Alasalvar et al., 2001) and/or submitted to different processing and storage conditions (Rodríguez-Sevilla et al., 1999; Nyman et al., 2005). However, the role of reducing sugars in the

MR has been scarcely studied in dehydrated carrots (Rufián-Henares et al., 2008). Recently, minor carbohydrates in carrots have been reported by Soria et al. (2009a) because of their remarkable role in a variety of biological functions.

Moreover, carrots are known as a good source of bioactive compounds such as natural antioxidants, including carotenoids, vitamins, phenolic compounds and flavonoids. Changes in several of these bioactives such as β-carotene, lycopene, etc, in carrots subjected to different drying techniques and the evolution of antioxidant capacity during the storage of selected fruits and vegetables have been previously studied (Regier et al., 2005; Hiranvarachat et al., 2008; Soria et al., 2009b).

Among the physical quality parameters, the rehydration ability is one of the most relevant parameters for the acceptance of dehydrated carrots by consumers. Conditions selected for pre-treatment, drying and rehydration, noticeably affect the structure and composition of carrot tissues (Stepien, 2008), which determine the organoleptic properties of carrots upon rehydration (Marabi et al., 2006).

On the other hand, with the aim of preserving the quality of dehydrated vegetables, several studies have been focused on the evaluation of the most relevant parameters involved in dehydration (Krokida et al., 2003a), the improvement of existing processes (Fernandes & Rodrigues, 2008) or the search for alternative or emergent technologies (Gallego et al., 2007). Among the latter, the use of power ultrasound (US) for the dehydration of vegetables has recently emerged as a novel alternative to conventional drying processes, with the advantages of mild treatment temperatures and short drying times (De la Fuente-Blanco et al., 2006). Although several papers have dealt with parametric and kinetic studies on moisture loss during the ultrasonic drying of carrots (García-Pérez et al., 2006a; 2009), no reference has yet addressed the physical, chemical and physico-chemical changes of vegetables during US-assisted drying.

The aim of this paper is to evaluate the quality and bioactivity of carrots processed under different US operating conditions, with a view to obtain premium quality dehydrated carrots. Our study also includes a comparison with a freeze-dried sample (used as a quality control) and data of commercial dehydrated carrots. To the best of our knowledge, this is the first time that

the evolution of the MR and changes in soluble sugars, proteins, total polyphenols, antioxidant activity and rehydration ability have been determined in US-dehydrated carrots.

Materials and methods

US-dehydrated carrot samples

Fresh carrots (*Daucus carota* L. var. Nantesa) were purchased from a local market in Madrid (Spain) and stored in the dark at 4 ºC within a maximum period of five days until dehydration. Experiments were carried out using a prototype of air-borne ultrasonic dehydration (De la Fuente-Blanco, 2006). The experimental setup mainly consists of (i) a hot-air generator, (ii) a stepped-plate power ultrasonic transducer with the corresponding electronic generator, (iii) a flat plate parallel to the ultrasonic radiator acting both as a reflector for the formation of a standing wave and as a sample holder, where suction is applied to remove the moisture, and (iv) a static pressure system to get good mechanical coupling between the carrot sample (16 slices: 24 mm diameter, 4 mm thickness) and the vibrating plate of the transducer.

In all experiments, ultrasonic parameters other than air temperature (20, 40 and 60 ºC) and drying time (75, 90 and 120 min) were kept constant: ultrasonic frequency = 20 kHz; power level = 100 W; air speed = 1.2 m s^{-1}; suction pump = 120 mbar and contact pressure 1.6 kg cm^{-2}. To evaluate the effect of sample pre-treatment on dehydration, carrots were blanched in boiling water for 1 min (ratio sample: water was 1:30) and were also processed under the different US experimental conditions detailed above (**Table 4.31**). Two replicates per set of dehydration conditions were carried out. For determinations other than the rehydration ability, samples were freeze-dried and finely ground using a thermostatized laboratory grinder (IKA A10, Jankie & Kunkel).

Table 4.31 Sample codes of carrots dehydrated by US

Carrot Sample	Dehydration conditions	
	Air temperature (ºC)	Drying time (min)
US20	20	120
US20BL[a]		120
US-40	40	90
US-40BL		90
US-60	60	75
US-60BL		75

[a]BL: carrots blanched prior to dehydration

Other dehydrated carrot samples

Six commercial dehydrated carrots (COMM1-6) from three different suppliers in Spain and a laboratory freeze-dried (FD) carrot were also analyzed. Samples were kept at -20 ºC until analysis.

Carrot characterization

The dry matter (DM) content was determined gravimetrically by drying the samples until constant weight (AOAC, 1990a). Total nitrogen (TN) was determined by the Kjeldahl method (AOAC, 1990b) and the protein level was calculated using 6.25 as conversion factor (TN × 6.25). All determinations were carried out in duplicate.

HPLC analysis of 2-furoylmethyl amino acid derivatives

The determination of 2-FM-AA was carried out by ion-pair RP-HPLC analysis (Resmini & Pellegrino, 1991) using a C_8 column (250 mm × 4.6 mm i.d.) (Alltech, Lexington, KY, USA) thermostated at 37 °C. A linear binary gradient (A: 4 mL L^{-1} acetic acid and B: 3 g L^{-1} KCl in A) at a flow rate of 1.2 mL min^{-1} was used. The elution programme was as follows: 100% A (from 0 to 12 min), 50% A (from 20 to 22.5 min) and 100% A (from 24.5 to 30 min). A variable-wavelength detector was set at 280 nm (LCD Analytical SM 4000).

Samples (0.25 g) were hydrolyzed under inert conditions (helium) with 4 mL 8 M HCl at 110 °C for 23 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolysate was filtered through a medium-grade paper filter (Whatman no. 40). 0.5 mL of the filtrate were applied to a Sep-Pack

C18 cartridge (Millipore, MA, USA) pre-wetted with 5 mL of methanol and 10 mL of water, then eluted with 3 mL of 3 M HCl and 50 μL were injected.

The identification of 2-FM-AA derivatives other than furosine was first carried out by comparing the retention times with data previously obtained for standards synthesized in our laboratory and analysed under identical experimental conditions (Sanz et al., 2001). The identity of 2-FM-derivatives was further confirmed by HPLC-MS following the method described by Del Castillo et al. (2002). Analyses were carried out at room temperature on a Hewlett-Packard 1100 liquid chromatograph coupled to a quadrupole HP-1100 mass detector (both from Hewlett-Packard, Palo Alto, CA, USA), working in electrospray ionization mode, under atmospheric pressure and positive polarity (API-ES positive). The mobile phase was acetic acid in Milli-Q water (20 mL L^{-1}) and elution was under isocratic conditions at a flow rate of 0.7 mL min^{-1}. Mass spectrometer values of needle potential, gas temperature, drying gas and nebuliser pressure were 4000 V, 350 ºC, 11 L min^{-1} and 55 psi, respectively. Scan range was 100-900 uma and the fragmentator potential was 60 V.

Quantitation was performed by the external standard method, using a commercial standard of 2-FM-lysine (furosine) (Neosystem Laboratoire, Strasbourg, France). All the analyses were performed in duplicate and the data shown in this paper are the mean values expressed as mg/100 g protein.

GC analysis of carbohydrates

Carrot soluble sugars were extracted in duplicate according to the method described by García-Baños et al. (García-Baños et al., 2000). 0.1 g of carrot samples were weighed into a 25-mL volumetric flask and extracted at room temperature with 5 mL of Milli-Q water for 20 min with constant stirring. The volume was made up to 25 mL with pure ethanol to obtain a final 80% ethanolic solution. Then, samples were centrifuged at 9,600 *g* and 10 ºC for 10 min. Precipitates were submitted to a second extraction with 25 mL of 80% ethanol to obtain recovery values close to 100%. 1 mL of supernatants was mixed with 0.2 mL of an ethanolic solution of phenyl-β-D-

glucoside (1 mg mL^{-1}; Sigma Chemical Co., St. Louis, US) used as internal standard. The mixture was evaporated under vacuum at 40 ºC.

GC analyses were performed with an Agilent Technologies 7890A gas chromatograph equipped with a flame ionisation detector (FID), using nitrogen as carrier gas. The trimethylsilyl oxime (TMSO) derivatives prepared, as described by Sanz et al. (2004), were separated using an HP-5 MS fused-silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) coated with 5% phenylmethylsilicone (J&W Scientific, CA, USA). The carrier gas flow rate was 1 mL min^{-1}. Oven temperature was held at 200 ºC for 11 min, then raised to 270 ºC at a heating rate of 15 ºC min^{-1}, then raised again to 300 ºC at 3 ºC min^{-1} and finally raised to 325 ºC at 15 ºC min^{-1}. The injector and detector temperatures were 280 and 325 ºC, respectively. Injections were made in the split mode (1:40).

Data acquisition and integration were performed using Agilent ChemStation Rev. B.03.01 software (Wilmington, USA). The identification of TMSO derivatives of carbohydrates was carried out by comparing the experimental retention indices with those of standards previously derivatized. Quantitative data (mg g^{-1} DM) were calculated from FID peak areas. Standard solutions of fructose, glucose, sucrose, *scyllo*- and *myo*-inositol (all of them from Sigma Chemical Co., St. Louis, USA) over the expected concentration range in carrot extracts were prepared to calculate the response factor relative to phenyl-β-D-glucoside. In the absence of any commercial standard, the concentration of sedoheptulose was estimated assuming a response factor equal to 1.

Preparation of protein isolates and analysis by SDS-PAGE

100 mg of dehydrated carrot powders were mixed with 2 mL of Milli-Q water containing 1% sodium metabisulfite (Merck, Darmstadt, Germany), and stirred thoroughly for 2 hs. The mixed slurry was centrifuged at 3,000*g* for 15 min, and the supernatant was analyzed by SDS-PAGE.

For SDS-PAGE analysis, 32.5 µL of sample supernatant were added to 12.5 µL of 4X NuPAGE® LDS Sample buffer (Invitrogen, CA, USA) and 5 µL of 0.5 M dithiothreitol (DTT, Sigma-Aldrich, St. Louis, USA) and heated at 70 ºC for 10 min. Samples (20 µL) were loaded on a 12% polyacrylamide

NuPAGE® Novex Bis-Tris pre-cast gel and a continuous MES SDS running buffer was used. Gels were run for 41 min at 120 mA/gel and 200 V and stained using the Colloidal Blue Staining Kit (Invitrogen, CA, USA). The molecular weight of proteins was estimated by using a mixture of standard proteins with relative molecular weights ranging 2.5-200 kDa (Invitrogen, CA, USA): myosin (200 kDa), ß-galactosidase (116.3 kDa), phosphorylase B (97.4 kDa), BSA (66 kDa), glutamic dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), aprotinin (6 KDa), insulin B chain (3.5 kDa), and insulin A chain (2.5 kDa).

Measurement of total phenolic content (TPC) by Folin-Ciocalteau method

Methanolic extracts were prepared by adding 2.5 mL of HPLC grade methanol to 0.1 g of dehydrated carrot powders and homogenising for 1 min at 24,000 rpm using an Ultra-Turrax® T-25 homogenizer (Janke and Kunkel, IKA Labortechnik, Saufen, Germany). The samples were stirred for 20 min at 750 rpm using a Thermomixer (Eppendorf, Germany) and centrifuged for 15 min at 2,000*g*. Supernatants were then filtered through PVDF Acrodisc syringe filters (0.45 µm, Sigma-Aldrich, St. Louis, USA) for subsequent analysis.

TPC in carrots was determined using Folin-Ciocalteau reagent (2N, Sigma) according to the method of Singelton et al. (1999) and Patras et al. (2009) with slight modifications. 100 µL of filtered methanolic extract, 100 µL of MeOH and 100 µL of Folin-Ciocalteau reagent were vortexed in a 1.5 mL eppendorf. After 5 min, 700 µL of 75 g L^{-1} Na_2CO_3 were added and the samples were vortexed briefly. The eppendorfs were then left in the dark for 20 min at room temperature. Following this, the samples were centrifuged at 13,000 rpm for 3 min. The absorbance of the sample was read at 735 nm using aqueous gallic acid (Sigma-Aldrich, St. Louis, MO, USA) 10-400 mg L^{-1} as standards. Results were expressed as milligrams of gallic acid equivalent (GAE)/g DM.

Antioxidant activity by the oxygen radical absorbance capacity (ORAC) assay

25 mg of dehydrated carrot powders accurately weighed were mixed with 1 mL of acetone/water (50:50, v/v) extraction solvent. The mixture was shaken at room temperature for 1 h. The extracts were centrifuged at 14,000 rpm for 15 min, and the supernatant was ready for analysis after appropriate dilution with 75 mM potassium phosphate buffer solution (pH 7.4) (Sigma-Aldrich, St. Louis, USA).

The ORAC assay using fluorescein as fluorescent probe was based on that proposed by Ou et al. (2001) and modified by Dávalos et al. (2004). Trolox, a water-soluble analogue of vitamin E, was used as a control standard. The reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) with a blank sample (no antioxidant) in parallel, and the final assay mixture (200 µL) contained fluorescein (116.6 nM), AAPH (48 mM), and antioxidant (10-80 µM Trolox or sample). All standards used for ORAC assay were purchased from Sigma-Aldrich (St. Louis, USA).

A micro assay based on the use of black 96-well microplates (96F untreated, Nunc, Denmark) was used for fluorescence measurements. The plate was automatically shaken before the first reading, and the fluorescence was recorded every min for 98 min after addition of AAPH. A Polarstar Galaxy plate reader (BMG Labtechnologies GmbH, Offenburg, Germany) with 485-P excitation and 520-P emission filters and controlled by the Fluostar Galaxy software version (4.11-0) was used. AAPH and Trolox solutions were prepared daily and fluorescein was diluted from a stock solution (1.17 mM) in 75 mM phosphate buffer (pH 7.4). All reaction mixtures were prepared in duplicate and the analysis for each sample was carried out in triplicate. Fluorescence measurements were normalized to the curve of the blank. From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as:

$$AUC = 1 + \sum_{i=1}^{i=98} f_i / f_0 \qquad (1)$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to a sample was calculated as follows:

$$\text{net AUC} = \text{AUC}_{\text{antioxidant}} - \text{AUC}_{\text{blank}} \qquad (2)$$

The regression equation between net AUC and antioxidant concentration was calculated. The slope of the equation was used to calculate the ORAC value by using the Trolox curve obtained for each assay. Final ORAC values were expressed as μmol of Trolox equivalent (TE)/g DM.

Rehydration ability

Carrot slices were rehydrated by immersion in distilled water (solid-to-liquid ratio 1:50) at room temperature for 24 h. After blotting with tissue paper to remove any superficial water, rehydrated carrots were weighed. Each rehydration experiment was performed in triplicate and no correction was made for lost solids.

Rehydration ratio (*RR*) (Lewicki, 1998b) was calculated as follows:

$$RR = \frac{m_r}{m_d} \tag{3}$$

where m_r is the mass of the rehydrated sample (g) and m_d is the weight (g) of the dehydrated carrot.

Leaching loss

Lost solids during rehydration were determined according to the AOAC method (1990a). The soak water was placed in a pre-weighted evaporating beaker and dried in a conventional oven at 102 ºC for 24 h. The residue was weighted and the percentage of leached solids (*LL*, %) with respect to the initial weight of dehydrated carrot was calculated.

Diameter change

The change in the carrot diameter during the rehydration process was measured using vernier callipers (Mitutoyo Corporation, Japan) and calculated according to Bhattacharya (1995):

$$d(\%) = \frac{d_r - d_d}{d_d} \quad (4)$$

where d is the carrot diameter increase during the rehydration process (%); d_r is the diameter of the rehydrated sample (mm) and d_d is the diameter of the initial dehydrated sample (mm).

Statistical analysis

Data were subjected to one-way analysis of variance (Tukey HSD Multiple Range Test) by applying the Statgraphic 4.0 Program (Statistical Graphics Corporation, Rockville, MD, USA) for Windows. The significance of differences was defined as $P < 0.05$.

Results and discussion

Carrot dehydration

Figure 4.23 shows the drying curves obtained in the dehydration of carrots by US under different operating conditions. As observed, moisture loss values higher than 85%, which assure the microbiological stability of dehydrated carrots, were obtained for the different conditions tested. In agreement with the results previously reported by other authors (De la Fuente-Blanco et al., 2006; García-Pérez et al., 2006b), the synergic effect of US and temperature increased the dehydration rate of carrots, with moisture loss rates up to 90% in 75 min for sample US-60BL. US technology produces a series of effects (microagitation, creation of microscopic channels and cavitation of water molecules) which make the moisture removal easier and allow dehydration to be carried out at milder temperatures (60 ºC or less) (Mulet et al., 2003), this being particularly useful for preserving the bioactivity of heat-sensitive carrot constituents.

Blanching also showed a positive effect on the dehydration rate of US-processed carrots at any set of operating conditions. It is well-known that high-temperature and short-time blanching has a beneficial effect not only on the inactivation of enzymes, which, if untreated, could be active at least

during the early stages of drying, but also on the shortening of drying times (Lewicki, 2006).

Regarding precision, relative standard deviation values in the range 0.1-0.3% show the excellent reproducibility of the US-dehydration process irrespective of the experimental conditions tested here.

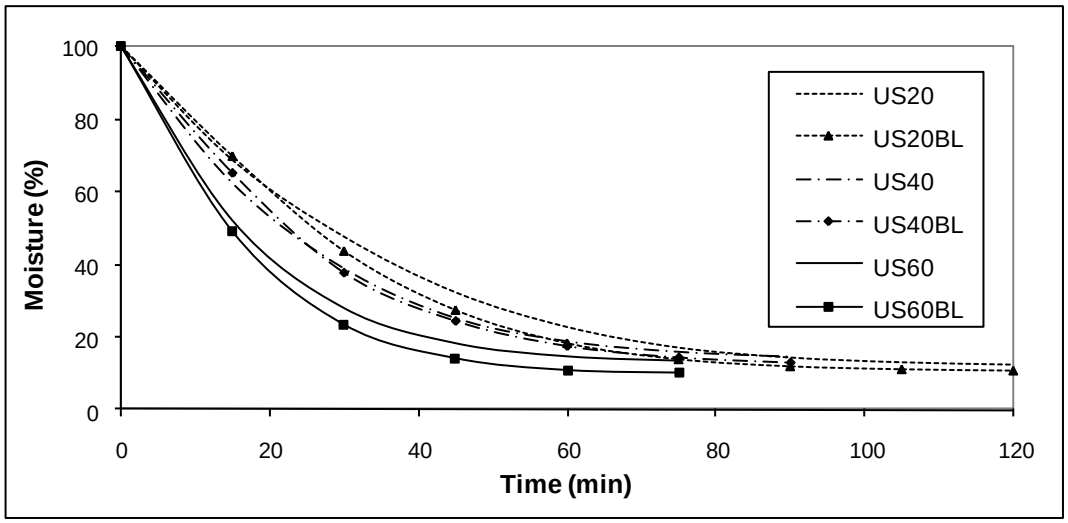

Figure 4.23 Drying curves of carrots dehydrated by US under different operating conditions. For nomenclature of samples, see Table 4.31.

Maillard reaction evolution

Figure 4.24 shows, as an example, the RP-HPLC chromatographic profile of 2-FM-AA obtained for the acid hydrolysates of three of the dehydrated carrots under analysis: FD, US-60BL and COMM2. Identification of 2-FM-AA of Lys, Arg, γ-aminobutyric acid (GABA) and Ala was confirmed by coinjection of the corresponding standards synthesised in our laboratory and by LC-MS analysis.

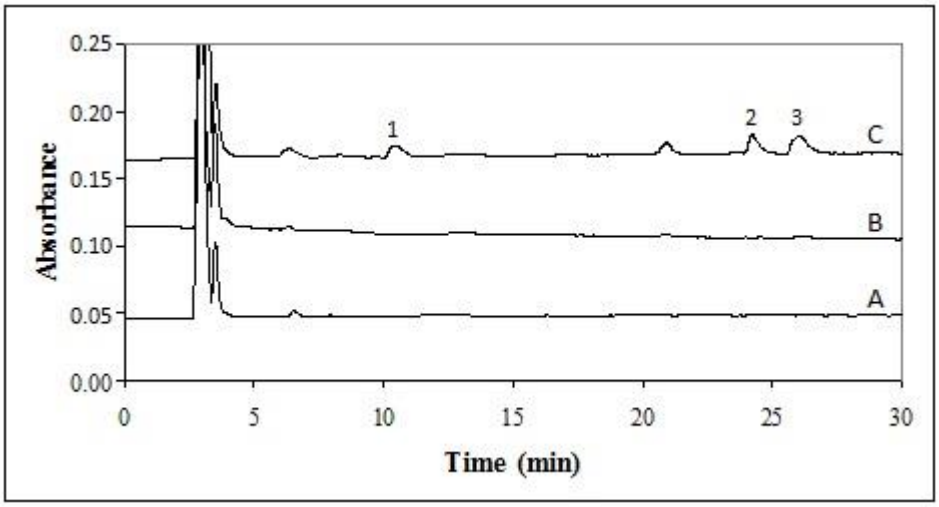

Figure 4.24 RP-HPLC-UV chromatogram of 2-FM-AA in acid hydrolisates of (A) freeze-dried carrot, (B) US dehydrated carrot (US-60BL), and (C) commercial dehydrated carrot (COMM2). (1) 2-FM-Ala, (2) 2-FM-GABA, and (3) 2-FM-Lys + 2-FM-Arg.

2-FM-Lys + 2-FM-Arg (peak 3 in **Figure 4.24**) were only detected in carrots dehydrated by power US at 60 ºC (**Table 4.32**). Samples subjected to blanching before US-processing presented a slightly higher level of 2-FM-Lys+2-FM-Arg as compared to samples with no pre-treatment, probably due to a higher dehydration rate of these samples (**Figure 4.23**). Similarly to the freeze-dried carrots, no formation of 2-FM-Lys+2-FM-Arg in carrots processed by US at 20 and 40 ºC was detected. Levels of this quality marker in carrots dehydrated by US at 60 ºC were significantly lower than those of commercial dehydrated carrots here analysed and data previously reported by Soria et al. (2009b) for industrially-dried carrots (average of 589 mg/100 g protein). Levels of 403 mg of 2-FM-Lys/100 g protein have also been reported by Rufián-Henares et al. (2008) in a carrot-based product dehydrated under mild temperature conditions (30 ºC for 180 h). The content of 2-FM-Ala and 2-FM-GABA (traces in US-dehydrated samples) was always lower than that of 2-FM-Lys + 2-FM-Arg, supporting thus the usefulness of the latter joint marker as a sensitive quality indicator to control the early stages of MR in carrots subjected to dehydration.

Table 4.32 Quantitative analysis of 2-FM-AA in dehydrated carrot samples (mean of 2 replicates ± SD)

CARROT SAMPLE	2-FM-AA (mg / 100 g protein)		
	2-FM-Lys + 2-FM-Arg	2-FM-GABA	2-FM-Ala
FD[1]	n.d.[2a]	n.d.[a]	n.d.[a]
FDBL[3]	n.d.[a]	n.d.[a]	n.d.[a]
US-60	23 ± 1[a]	tr[2a]	n.d.[a]
US-60BL	39 ± 1[a]	tr[a]	n.d.[a]
COMM1	848 ± 49[b]	312 ± 10[b]	98 ± 6[b]
COMM2	447 ± 11[c]	279 ± 16[bc]	216 ± 34[c]
COMM3	426 ± 18[c]	228 ± 19[c]	119 ± 10[b]
COMM4	819 ± 102[b]	599 ± 65[d]	618 ± 82[d]
COMM5	416 ± 18[c]	312 ± 6[b]	154 ± 3[bc]
COMM6	358 ± 14[c]	152 ± 6[e]	134 ± 1[bc]

[1]FD: Freeze-dried carrot
[2]n.d.: not detected; tr: traces
[3]BL: carrots blanched prior to dehydration
a-e: samples with the same superscript letter within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Carbohydrate analysis

Table 4.33 lists the concentration of major and minor soluble carbohydrates determined in carrots experimentally dehydrated by US or freeze-drying and in six commercial dehydrated carrots. In agreement with the evolution of the MR early stages, the content of reducing sugars (fructose and glucose) showed the highest change for blanched samples processed by US at 60 ºC; this decrease (54%) being particularly noticeable for glucose due to its higher involvement in MR. A very low decrease in the content of glucose and fructose was observed in samples US20 and US-40, as compared to data of US-60. These results seem to indicate the slight effect of US processing on reducing carbohydrates at low temperature. Hardly any change was found for sucrose and minor carbohydrates in carrots processed by power US at different operating conditions.

Regarding the effect of blanching, no significant differences associated with the loss of sugars by lixiviation were found for freeze-dried samples FD and FDBL. However, the probably higher porosity of blanched samples, which could favour the diffusion of water and the most soluble carbohydrates to the surface, might contribute, among others, to their loss during US dehydration.

Major sugars showed a wide variability in commercial dehydrated carrots; the content here determined for samples COMM1-COMM6 falling in

the range previously reported for other dehydrated carrots (Rodríguez-Sevilla et al., 1999; Soria et al., 2009a; 2009b). Inositols, such as *scyllo*- and *myo*-inositol, naturally present in several food products of vegetable origin, have been reported to be stable during the different stages of convective air drying of carrots (Soria et al., 2009b) and in orange juice subjected to different storage and processing conditions (Villamiel et al., 1998). In agreement with this, results listed in **Table 4.33** show the low variability in the concentration of these two compounds irrespective of the processing conditions (US, freeze-drying) or the commercial sample considered (COMM1-6). However, a wider range of variation was found for sedoheptulose, a minor carbohydrate described for the first time in carrots by Soria et al. (2009a). Whereas average concentration of sedoheptulose in US-dehydrated carrots (16 mg g^{-1} DM) matched well that of freeze-dried carrots (16.7 mg g^{-1} DM), it was higher than that of most commercial carrots (8-11 mg g^{-1} DM except for COMM4 showing 27 mg g^{-1} DM). The different variety or degree of ripeness of carrots subjected to dehydration could be responsible for the differences observed. Losses of minor carbohydrates due to blanching were lower than those of the more water-soluble major sugars.

Table 4.33 Quantitative analysis of carbohydrates in dehydrated carrot samples under analysis (mean of 2 replicates ± SD)

CARROT SAMPLE	Carbohydrates (mg g^{-1} DM) ± SD					
	Fructose	Glucose	Sucrose	Sedoheptulose	*Scyllo*-inositol	*Myo*-inositol
FD[1]	79.7 ± 0.6^{a}	76.7 ± 4.3^{a}	493.6 ± 11.1^{a}	16.7 ± 0.8^{ab}	2.2 ± 0.1^{abc}	2.9 ± 0.2^{a}
FDBL[2]	75.1 ± 1.5^{b}	74.4 ± 1.6^{a}	465.5 ± 16.2^{ab}	15.6 ± 1.3^{abc}	1.9 ± 0.1^{abd}	2.6 ± 0.01^{a}
US20	74.2 ± 2.2^{bc}	62.9 ± 3.3^{ab}	470.5 ± 21.2^{ab}	19.1 ± 0.8^{b}	2.7 ± 0.2^{ce}	3.5 ± 0.6^{ab}
US20BL	51.2 ± 10.5^{d}	41.0 ± 9.9^{cde}	422.6 ± 11.1^{bc}	16.4 ± 0.3^{abc}	2.0 ± 0.2^{abcd}	3.1 ± 0.3^{ab}
US-40	73.8 ± 1.4^{abc}	62.5 ± 3.7^{ab}	458.9 ± 17.3^{ab}	13.7 ± 0.3^{abcde}	1.5 ± 0.3^{df}	2.6 ± 0.1^{a}
US-40BL	49.9 ± 2.0^{d}	39.1 ± 1.1^{cde}	443.1 ± 17.4^{ab}	15.3 ± 0.9^{abc}	1.2 ± 0.07^{f}	2.5 ± 0.1^{a}
US-60	51.7 ± 3.1^{cd}	45.7 ± 1.7^{bde}	489.6 ± 8.7^{a}	17.6 ± 0.7^{ab}	2.2 ± 0.2^{abc}	3.4 ± 0.2^{ab}
US-60BL	45.6 ± 12.5^{de}	34.1 ± 8.3^{f}	436.8 ± 5.6^{b}	14.9 ± 3.4^{abce}	2.2 ± 0.1^{abc}	3.5 ± 0.5^{ab}
COMM1	47.8 ± 2.8^{de}	39.9 ± 2.4^{cde}	347.9 ± 8.9^{de}	8.8 ± 0.4^{df}	1.7 ± 0.2^{adf}	3.0 ± 0.2^{a}
COMM2	41.3 ± 0.2^{de}	21.9 ± 0.2^{cf}	347.2 ± 0.3^{de}	7.5 ± 0.1^{f}	2.2 ± 0.1^{abc}	4.0 ± 0.02^{bc}
COMM3	47.3 ± 1.9^{de}	35.5 ± 2.4^{cdf}	375.2 ± 12.0^{ce}	10.0 ± 0.7^{def}	2.4 ± 0.2^{bc}	4.8 ± 0.2^{c}
COMM4	85.2 ± 4.5^{a}	58.3 ± 4.1^{abe}	314.0 ± 13.6^{d}	27.1 ± 0.7^{g}	3.2 ± 0.2^{e}	4.0 ± 0.1^{bc}
COMM5	57.1 ± 0.9^{bcd}	48.6 ± 1.5^{bde}	434.2 ± 13.0^{b}	11.4 ± 0.02^{cdef}	2.6 ± 0.05^{ce}	4.7 ± 0.1^{c}
COMM6	27.2 ± 0.3^{e}	16.3 ± 1.4^{f}	447.1 ± 5.1^{ab}	8.7 ± 0.3^{df}	3.0 ± 0.1^{e}	5.0 ± 0.04^{c}

[1]FD: Freeze-dried; [2] BL: carrots blanched prior to dehydration.
a-f: samples with the same superscript letter within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

SDS-PAGE analysis of carrot proteins

As a consequence of the dehydration process, cross-linking and aggregation of proteins may occur, modifying their structure and, consequently, their functionality. However, and to the best of our knowledge, no studies on the evaluation of the changes in the structure of carrot proteins following dehydration by power US have been carried out so far. With this purpose, the electrophoretic profiles of FD, COMM4, COMM6, US-60 and COMM1 samples were obtained by SDS-PAGE under reducing conditions. FD carrots showed four major bands with molecular weight of ~ 18, 36.5, 41.2 and 55.4 kDa (**Figure 4.25**, lane 1). All commercial samples (**Figure 4.25**, lanes 2, 3 and 5) analysed showed different patterns of bands as compared to FD. According to the results derived from the analysis of the acid hydrolysates of the Amadori compounds (**Table 4.32**), bands observed in commercial samples could be attributed to unfolding, cross-linking and aggregation of proteins taking place during the advanced stages of the MR. This is particularly noticeable in sample COMM4 (**Figure 4.25**, lane 2), which showed a variety of bands with slower electrophoretic mobility and different molecular weight, indicative of the formation of a wide range of glycated species of proteins.

In contrast, a similar pattern to that of FD was observed for US-60 (**Figure 4.25**, lane 4), indicating that ultrasonic drying does not cause important structural changes in carrot proteins, as supported by the limited extent of the MR in US-60 when compared to the commercial samples analysed (**Table 4.32**).

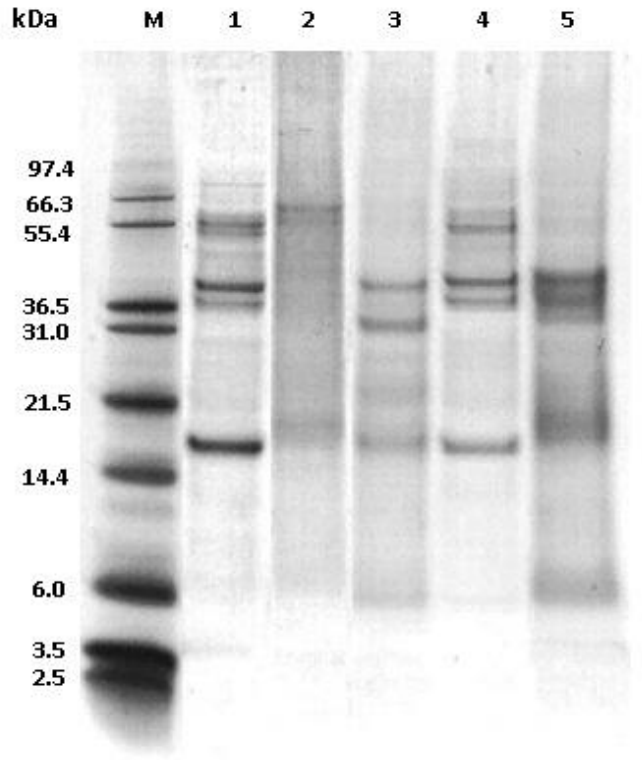

Figure 4.25 SDS-PAGE analysis of dehydrated carrots: (1) FD, (2) COMM4, (3) COMM6, (4) US-60, and (5) COMM1. (M) Markers of molecular weight.

Total phenolic content and antioxidant activity

As previously described, the antioxidant activity of carrots is due to different compounds, including *β*-carotene, vitamin C, polyphenols, etc. Solvent extraction is usually employed for isolation of antioxidants and both extraction yield and activity of extracts are strongly dependent on the solvent, due to the different antioxidant properties of compounds with different polarity extracted (Moure et al., 2001). Therefore, two carrot extracts for TPC and ORAC in solvents of different polarity were prepared as described under *Materials and Methods*.

Firstly, the total phenolic content (TPC) of carrot methanolic extracts was determined by the Folin-Ciocalteau method (**Table 4.34**). Similar results were obtained for all the laboratory-dried carrots, irrespective of the dehydration technique (US or freeze-drying). The longer processing time in US-40 and higher processing temperature in US-60 as compared to US20 could be responsible for the slight decrease in polyphenol content of these samples. A similar effect has been reported by Chantaro et al. (2008) for

other thermolabile antioxidants such as β-carotene in carrot peels subjected to drying at temperatures of 60 and 70 ºC (shorter drying times at higher temperatures decrease the degradation reaction). Changes in physical properties such as texture, matrix softening, etc in carrots processed at different drying conditions may also affect the extractability of antioxidants and, therefore, their bioactivity (Gorinstein et al., 2009).

Table 4.34 Total phenolic content (TPC) and antioxidant activity by ORAC assay of dehydrated carrots under analysis (mean of 2 replicates ± SD)

CARROT SAMPLE	TPC mg GAE/g DM	ORAC µmol TE/g DM
FD [1]	1.365 ± 0.081^{a}	31.22 ± 0.606^{a}
US20	1.366 ± 0.046^{a}	21.82 ± 0.075^{c}
US20BL[2]	1.331 ± 0.078^{ac}	24.49 ± 0.030^{b}
US-40	1.111 ± 0.023^{b}	19.09 ± 0.016^{d}
US-40BL	1.101 ± 0.011^{b}	24.33 ± 0.189^{b}
US-60	1.235 ± 0.039^{abc}	24.43 ± 0.548^{b}
US-60BL	1.252 ± 0.080^{abc}	25.41 ± 0.385^{b}
COMM1	1.628 ± 0.056^{d}	29.57 ± 0.460^{a}
COMM2	2.885 ± 0.049^{e}	56.38 ± 0.955^{e}
COMM3	1.931 ± 0.011^{f}	45.97 ± 0.893^{f}
COMM4	3.246 ± 0.065^{g}	53.91 ± 0.572^{e}
COMM5	1.651 ± 0.006^{d}	36.52 ± 0.743^{g}
COMM6	1.680 ± 0.072^{d}	38.40 ± 0.374^{g}

[1] FD: Freeze-dried
[2] BL: carrots blanched prior to dehydration
a-g: samples with the same superscript letter within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Antioxidant activity was determined by ORAC assay in acetone/water extracts. Drying temperature in the 20-60 ºC range did not significantly affect the results of this assay; all of them being slightly lower than that of the FD sample. The high correlation (R^2 = 0.8662) between TPC and ORAC measurements for all the samples under analysis suggest that the presence of phenolic compounds largely accounted for their antioxidant capacity. Similar results were found by Zhang and Hamauzu (2004) who found that antioxidant and radical scavenging activities in different carrot tissues decreased in the same order as the phenolic content.

Results of the ORAC assay listed in **Table 4.34** for both commercial and laboratory-dehydrated samples fall well in the range reported for freeze-dried

carrots collected from various USA marketplaces at different harvesting seasons (25-99 μM TE g^{-1} DM). As for phenolic compounds, the differences observed between commercial and laboratory-dried carrots are supposed to be mainly originated from the dependence of carrot bioactivity on its variety, geographical origin, harvest time and processing conditions (Alasalvar et al., 2001; Gorinstein et al., 2009). Although the higher antioxidant activity of commercial samples could also be related to the higher MR evolution (**Table 4.32**), which might probably give rise to the formation of advanced glycation end products (AGEs) with antioxidant activity (Moreno et al., 2006), it is not possible to confirm this possibility due to the lack of information on the processing conditions of these samples. The effect of storage, which is deemed to play an important role on the antioxidant capacity and phenolics of different fresh fruits and vegetables, might also contribute to these differences (Kevers et al., 2007).

Contradictory results have been reported on the effect of a previous blanching on the preservation of the bioactive compounds and the antioxidant activity of different vegetables (Chantaro et al., 2008; Gorinstein et al., 2009). In this study, while blanching showed no influence on the TPC of the samples undergoing US drying under identical operating conditions, the antioxidant potential was slightly increased with sample pre-treatment before drying for samples processed at 20 and 40 ºC.

Rehydration ability

A significant improvement in the rehydration ability of carrots processed by US was observed for blanched samples (**Table 4.35**). It has been reported that the loosening of the cellular network and the separation along the middle lamella observed after blanching, result in a softening of the carrot tissue. Moreover, a reduced cohesiveness of the matrix improves water absorption and yields better rehydrated products (Lewicki, 2006). Blanching has also been reported to modify the structural characteristics of fiber, hence facilitating the water uptake of carrot peels (Chantaro et al., 2008).

Table 4.35 Rehydration Ratio (RR), Leaching Loss (LL, %) and diameter change (d, %) after rehydration of carrot samples under analysis (mean of 3 replicates ± SD)

CARROT SAMPLE	RR	LL (%)	d (%)
FD[1]	6.36 ± 0.02^{abc}	44.96 ± 2.06^{ab}	--
FDBL[2]	6.71 ± 0.95^{bcd}	50.49 ± 1.40^{abcd}	--
US20	4.66 ± 0.20^{e}	51.26 ± 1.09^{abcd}	24.67 ± 2.87^{a}
US20BL	8.00 ± 0.09^{d}	44.49 ± 1.58^{ab}	41.89 ± 2.05^{b}
US-40	4.87 ± 0.11^{ae}	52.79 ± 0.49^{bcd}	28.22 ± 0.03^{ac}
US-40BL	7.96 ± 0.26^{cd}	46.94 ± 5.21^{ab}	45.87 ± 4.46^{b}
US-60	4.92 ± 0.16^{ae}	55.30 ± 0.44^{cd}	21.00 ± 1.84^{a}
US-60BL	8.04 ± 0.50^{d}	46.60 ± 2.62^{abc}	39.70 ± 1.75^{bc}
COMM1	6.22 ± 0.20^{ab}	55.87 ± 2.68^{bcd}	--
COMM2	5.69 ± 0.16^{abe}	55.34 ± 2.52^{d}	--
COMM3	7.20 ± 0.31^{bcd}	43.86 ± 1.50^{a}	--
COMM4	6.25 ± 0.83^{abe}	49.49 ± 5.52^{abcd}	--
COMM5	5.97 ± 0.14^{abe}	53.01 ± 0.69^{bcd}	--
COMM6	6.47 ± 0.01^{abcd}	51.30 ± 2.96^{abcd}	--

[1]FD: Freeze-dried

[2]BL: carrots blanched prior to dehydration

a-e: samples with the same superscript letter within the same column showed no statistically significant differences for their mean values at the 95.0% confidence level.

Rehydration ratios in the range 5.69-7.20 were found for samples COMM1-COMM6; this means that the structural damage and cell shrinkage occurred during the drying process of these samples were higher than those of the blanched carrots submitted to US dehydration (*RR* ~ 8). The development of greater internal stresses and the creation of pores which facilitate the water uptake contributed to the higher *RR* observed for US-dehydrated carrots. Blanched carrots dehydrated by US also showed a better rehydration ability than the freeze-dried carrots here analysed (considered as a reference of high quality dehydrated carrots) and those previously subjected to citric acid or NaCl treatment to improve their rehydration properties, with *RR* in the range of 3-7 (Curry et al., 1976*;* Zambrano et al., 2007).

As a consequence of blanching, the loss of soluble solids and the solubilization of structure polymers such as protopectin may take place (Chantaro et al., 2008). Average leaching losses of 50% were found for both US dehydrated and commercial carrots. No significant improvement in the

leaching loss (%) was found for samples subjected to blanching prior to US dehydration.

In addition, the visual appearance of dehydrated products after rehydration is of prime importance for the consumers' acceptance of the product. Therefore, the change in diameter after rehydration was measured for carrots submitted to US dehydration (**Table 4.35**). As expected, a high correlation was found between the results of *RR* and *d* (%), with blanched samples showing a similar appearance to that of raw carrots (**Figure 4.26)**.

Figure 4.26 Rehydration of ultrasound-assisted hot air-dried carrot. Visual aspect before (A) and after (B) rehydration.

Conclusions

The preservation of the quality and bioactivity of carrots dehydrated by power US has been evaluated for the first time by measuring the evolution of the MR and the changes in soluble sugars, proteins, total polyphenols, antioxidant activity and rehydration ability. The effect of conventional blanching (high temperature, short time) prior to US dehydration of carrots has also been evaluated.

Power US not only improves the rate of dehydration as compared to conventional processes, but the milder processing conditions used in US drying also limit the MR extent in dehydrated carrots. Minor changes in reducing sugars, total phenolic content, antioxidant activity and similarity of protein profiles for both freeze-dried and ultrasonically dried samples, together with improved rehydration properties of blanched carrots also

support US as an alternative to freeze-drying for obtaining dehydrated carrots of premium quality. Further studies should be carried out to evaluate the potential of US as a profitable alternative to freeze-drying for obtaining dehydrated carrots with enhanced quality.

4.3.1.2 Deshidratación sin contacto

4.3.1.2.1 Efecto de los ultrasonidos de potencia en el secado convectivo de fresa

Effect of power ultrasound on the convective drying of strawberry

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Journal of Food Engineering (en redacción)

Abstract

The application of power ultrasound as a way to improve the convective drying of strawberry has been assessed. The applied acoustic energy (30 and 60 W) and temperature (40-70 ºC) gave rise to a significant improvement of drying time (13-44%). Taking into account the external resistance to water transport and the shrinkage, diffusional models of drying kinetic were described. The application of power ultrasound involved a significant ($p<0.05$) improvement of the effective moisture diffusivity and the mass transfer coefficient, being less intense the effect at high temperatures. The results here reported highlight that ultrasonic application during convective drying is a promising supporting technology to reduce the drying time for a heat sensitive material as strawberry.

Introduction

Along many decades, the convective drying using hot air has been considered the traditional dehydration method for foodstuffs since it provides long shelf life and facilitates transportation and storage of the dry material at low cost. Despite being the technique most addressed, drying is considered one of the most energy intensive industrial operations. It is estimated that thermal dehydration processes account up to 25% of the industrial energy consumption in developed countries (Chen & Mujumdar, 2008).

In order to understand the drying process and be able to improve it, mass transfer phenomena has been studied taking into account the

controlling resistances (Bon et al., 2007; Giner, 2009; Ozuna et al., 2011; Barati & Esfahani, 2013). Water transfer is mainly controlled by the rate of the water movement inside the materials (internal resistance) and the convective transport from the solid surface to the air (external resistance, ER). The internal resistance is characteristic of the food material, while the external one depends mostly on the thickness of the diffusion boundary layer (Cárcel et al., 2007b). Despite the great efforts to improve the drying process, it is known that optimal requirements for the heat and mass transfer do not necessarily assure optimal quality of the final products.

During the conventional drying, the product quality loss is linked to the high temperatures and long drying times used. Thus, the limitations in conventional drying process could be partially overcome with the use of additional energy sources, such as microwave (Li et al., 2011), infrared radiation (Rastogi, 2012) or power ultrasound (US) (Cárcel et al., 2012; Chandrapala et al., 2012). These technologies can be divided into two groups, those that produce heating effects and those that their effects are mechanical. The former (microwave or infrared radiation) have a serious limitation due to the risk of product overheating, which could reduce its overall quality and processes must be carefully controlled to avoid a negative impact in dried products.

On the contrary, US waves produce mainly mechanical effects and their application can intensify the water removal without using a high amount of thermal energy during drying (Riera et al., 2011). This represents a great improvement in the field of environmental friendly and energy saving technologies, being US technology recognized as a good example to assure sustainability (Gallego-Juarez, 2010). The low thermal effect of power US application in gas media makes easier its application in drying heat-sensitive materials (Chemat et al., 2011; Awad et al., 2012; Cárcel et al., 2012). The ultrasonic effects in solid media are mainly linked to the rapid series of alternative compressions and expansions promoted by the ultrasonic waves in both the particle ("sponge effect") and the surrounding air. This mechanical force can create microscopic channels that allow an easier inner water movement (De la Fuente et al., 2006), as well as microstirring and high turbulence in the interfaces (Cárcel et al., 2012). Additionally, cavitation phenomenon could provoke the removal of the water molecules most

strongly attached (Soria & Villamiel, 2010). In gas-solid applications, such as drying US assisted without contact, a great difficulty exists to efficiently transfer the acoustic energy from the transducer to the air and then to the solid material, due to the high acoustic impedance mismatch and the energy absorption by air at US frequencies. In addition, product characteristics also affect the ultrasonic influence on drying processes (Ozuna et al., 2011). Previous works have addressed the influence of power US application in mass transfer during drying of potatoes (Ozuna et al., 2011), carrots (Cárcel et al., 2011), lemon (García-Pérez et al., 2009), orange peel (Ortuño et al., 2010; García-Pérez et al., 2012a) and olive leaves (Cárcel et al., 2010). However, to the best of our knowledge, no previous works have been carried out on the ultrasonically assisted convective drying of berries. In the case of strawberries are fruits with high nutritive value and bioactivity (Giampieri et al., 2012) and one of the largest fruit crops (Doymaz, 2008b). Therefore, the aim of this paper was to evaluate the influence of the air temperature and the applied ultrasonic energy on the convective drying of strawberry. Modeling was used as a necessary tool for quantifying the observed effect of the process variables.

Materials and Methods

Samples

Fresh strawberries (*Fragaria x ananassa* Duch) were purchased from a local market in Valencia (Spain) and stored at 5 ºC for a maximum of 3 days until drying. After washing in tap water, draining with blot paper and removing the external impurities, strawberries were cut into 2.5 ± 0.5 mm thickness slices along their longitudinal axis.

Moisture content

The moisture content of fresh strawberries was determined at 70 ºC and 80 mbar vacuum levels until constant weight (AOAC, 1990c).

Airborne US dryer

Strawberries were dried by using an ultrasonic-assisted convective dryer (**Figure 4.27**). The prototype was initially a current pilot-scale convective dryer, afterwards modified its drying chamber to generate US waves (García-Pérez et al., 2006a; Riera et al., 2011). The ultrasonic device includes a cylindrical vibrating radiator driven by a piezoelectric transducer (21.8 kHz), which generates a high-intensity ultrasonic field in the air medium, where the samples are placed. A high power US generator, an impedance matching unit and a digital power meter (WT210, Yokogawa Electric Corporation, Japan) regulate and measure the electrical parameters of the acoustic signal (voltage, intensity, phase, frequency and power). The air parameters (velocity and temperature) were controlled through a PID algorithm and a PC supervised the whole drying process.

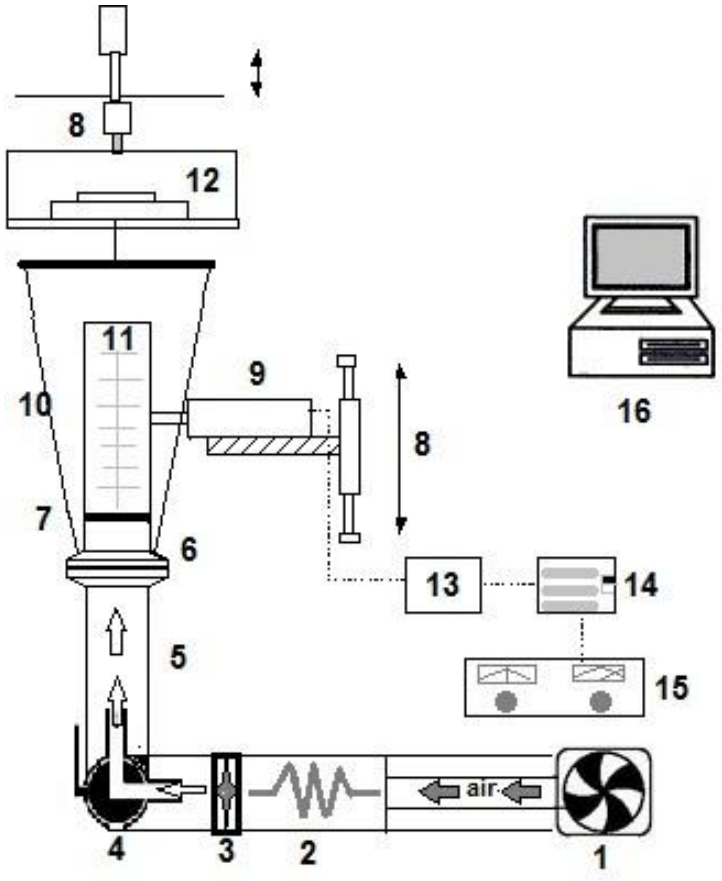

Figure 4.27 Diagram of the ultrasonic assisted drier. 1. Fan, 2. Heating unit, 3. Anemometer, 4. Three-way valve, 5. Thermocouple, 6. Sample loading chamber, 7. Coupling material, 8. Pneumatic system, 9. Ultrasonic transducer, 10. Vibrating cylinder, 11. Trays, 12. Balance, 13. Impedance matching unit, 14. Digital power meter, 15. High power ultrasonic generator, 16. PC.

Drying assays

An air velocity of 2 m/s was chosen for drying experiments of strawberries slabs, according to previous works carried out in the same dryer (García-Pérez et al., 2006a; 2009; Cárcel et al., 2007b). The temperature range was laid between 40 and 70 ºC (**Table 4.36**), which could be considered mild drying temperatures. Two levels of ultrasonic energy were set by applying specific electric powers to the transducer (30 and 60 W). Convective drying experiments were driven under the same experimental conditions but without applying US (0 W), thus being noted as "*nonUS*" drying experiments in the following sections. At least, three replicates of each

experimental condition were carried out. A summary of the processing conditions tested is shown in **Table 4.36**.

Samples (73.5 ± 3.5 g) were randomly distributed into the drying chamber to avoid the influence of the heterogeneity of the acoustic field generated in the results. For that purpose, samples were introduced in the vibrating chamber suspended in a metallic frame that allows free air flow around each individual piece. Sample weight was automatically recorded at 3 min intervals during the whole drying process.

Table 4.36 Processing conditions for ultrasonically assisted convective drying of strawberries

Assays	Temperature (ºC)	US power* (W)
nonUS-40	40	0
US-40-30	40	30
US-40-60	40	60
nonUS-50	50	0
US-50-30	50	30
US-50-60	50	60
nonUS-60	60	0
US-60-30	60	30
US-60-60	60	60
nonUS-70	70	0
US-70-30	70	30
US-70-60	70	60

*Specific electric power applied to the transducer.

Desorption isotherm

Water desorption isotherm were obtained from homogenized fresh strawberries. Samples (3.08 ± 0.05 g) were partially dehydrated in a conventional air-forced oven at 50 ºC for different times (up to 17 h) to ensure a wide range of final water content (0.2-11.2 kg water (W)/kg dry matter (DM)). The partial dried samples were kept at 25 ºC during 24 h in closed containers to achieve a homogeneous moisture distribution. Then, the water activity (a_w) measurement was carried out in a standardized conductivity hygrometer NOVASINA TH-500 (Air Systems for Air Treatment, Pfäffikon, Switzerland) at 25 ºC. The device was previously calibrated using the following salts: LiCl, $MgCl_2$, $Mg(NO_3)_2$, NaCl, $BaCl_2$ and $K_2Cr_2O_7$ according to the calibration procedure of the equipment manufacturer. Once a_w was

determined, sample moisture content was measured in triplicate (AOAC, 1990c). A total number of 30 water activity/moisture content experimental points were obtained. The well-known Brunauer, Emmet and Teller (BET, 1938) sorption isotherm model was the equation used to describe the relationship between experimental data of a_w and moisture (Eq. 1). The BET model was fitted to the experimental data by using the SOLVER optimization tool available in Microsoft EXCEL™, being identified the model parameters (the monolayer moisture content, W_m and the energy constant, C), which minimized the sum of the squared difference between experimental and calculated moisture content.

$$W = W_m \frac{Ca_w}{(1-a_w)(1+(C-1)a_w)} \quad (1)$$

Shrinkage determination

The product shrinkage determination was carried out with strawberries cubes (8.5 mm) dried at 70 ºC, using an air velocity of 2 m/s and without US application (0 W). During drying, three samples were randomly collected and weighed every 30 min, being measured moisture content (AOAC, 1990c) and volume. The toluene displacement method was used to measure the volume (toluene density 0.867 g/mL at 20 ºC) using a volumetric standard picnometer (48.89 mL) and an analytical balance (PB 303-5, Mettler Toledo) (García-Pérez et al., 2011). From the measurement of the volume, the length of the mass transport characteristic direction (L), which coincides with the half-length of the cube side, was calculated considering samples kept cubic geometry.

Modeling

The diffusion theory was considered to describe the water transfer phenomena during drying. The governing equation (Eq. 2) takes into account the solid isotropy and a constant effective moisture diffusivity (D_e) during drying (Simal et al., 2003).

$$\frac{\partial W_p(x,t)}{\partial t} = D_e \frac{\partial^2 W_p(x,t)}{\partial x^2} \quad (2)$$

Model solution was addressed considering that sample volume did not remain constant during drying due to the shrinkage phenomenon, which is especially noticeable in high-porosity products, such as fruits and vegetables (Schössler et al., 2012b). Thus, mass transport was addressed as a moving boundary problem considering the half length of the infinity slab (*L,* m) to be moisture dependent, which was experimentally determined as explained in above. For initial and boundary conditions, a homogeneous moisture content distribution in the solid (Eq. 3) and the solid symmetry (Eq. 4) was considered. Moreover the external resistance (ER) to mass transfer was taken into account as significant (Eq. 5) due to the low air velocity used and according to previous works reported in literature (García-Pérez et al., 2009).

$$t = 0 \qquad W_p(x,0) = W_0 \quad (3)$$

$$t > 0; x = 0 \qquad \frac{\partial W_p(0,t)}{\partial x} = 0 \quad (4)$$

$$t > 0; x = L \qquad -D_e \rho_{ds} \frac{\partial W_p(L,t)}{\partial x} = k(a_w(L,t) - \varphi_{air}) \quad (5)$$

where W_p is the local moisture content (kg W/kg DM), ρ_{ds} is the dry solid density (kg DM/m^3), k is the mass transfer coefficient (kg W/m^2/s), a_w is the a_w in the solid surface and φ_{air} is the relative humidity of the drying air. Eq. (2) was solved considering the initial and the boundary conditions already depicted by applying an implicit finite difference method (Mulet et al., 2005). For that purpose, a programming code was written in Matlab R 2009d (The MathWorks, Inc., Natick, MA), which provided the local moisture distribution

in the slab as well as the average moisture content (*W*). The model was fitted to the experimental drying kinetics by using the optimization tool *fminsearch function* (SIMPLEX method) available in Matlab. Thus, the D_e and k were simultaneously identified by minimizing the sum of the squared differences between the experimental and the calculated average moisture content.

In order to evaluate the fit of the models, the explained variance (VAR) and the mean relative error (MRE) were computed from Eq. (6) and (7) (Cárcel et al., 2011).

$$VAR = \left[1 - \frac{S_{tw}^2}{S_w^2}\right] \times 100 \tag{6}$$

$$MRE = \frac{100}{N}\left[\sum_{i=1}^{N} \frac{|W_{ei} - W_{ci}|}{W_{ei}}\right] \tag{7}$$

where S_w^2 and S_{tw}^2 are the variance of the experimental moisture data and the estimation, respectively, W_{ei} and W_{ci} are the experimental and calculated average moisture contents and N is the number of experimental data.

The Arrhenius equation was used (Sablani & Rahman, 2008) in order to quantify the influence of the temperature in the D_e values (Eq. 8).

$$D_e = D_0 \exp\left(\frac{-E_a}{RT}\right) \tag{8}$$

Where D_0 is the pre-exponential Arrhenius factor (m^2/s), E_a is the activation energy (kJ/mol), R is the universal gas constant (kJ mol/K), and T is the temperature (K).

Analysis of variance (ANOVA) and Least Significance Intervals (LSD) (Statgraphics 5.1 software) were carried out to identify the significance (95%) of the influence of factors of US application and air temperature on D_e and k.

Results and discussion

Experimental drying data

The strawberries presented an average initial moisture content of 9.55 ± 0.27 kg W/kg DM, which was considered as the critical moisture content due to the lack of constant rate period at these experimental conditions. **Figure 4.28** shows the effect of temperature and power US on the experimental drying kinetics.

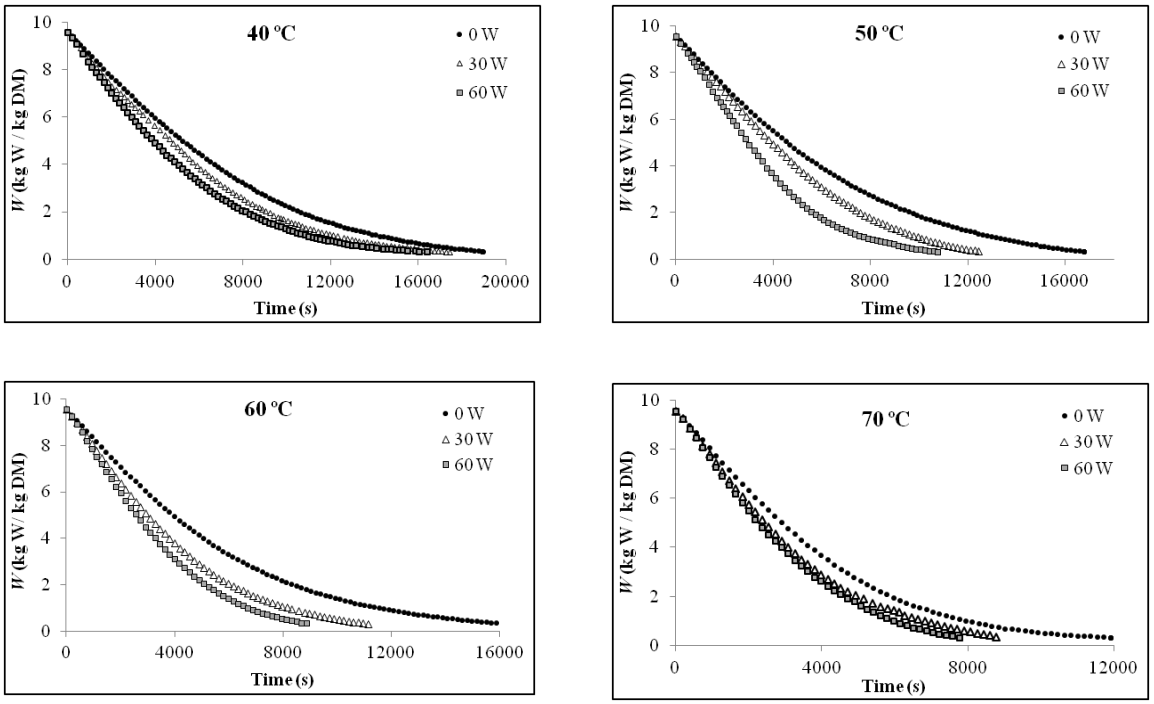

Figure 4.28 Drying kinetics of strawberry slabs (2 m/s, 40-70 ºC) applying different ultrasonic powers.

The increase of temperature gave rise to a substantial shortening of drying times, which was evident at all the US powers applied. Thus, samples dried without applying US at 40 ºC needed 5.3 h to reach an average moisture content of 0.3 kg W/kg DM, 4.6 h at 50 ºC, 4.4 h at 60 ºC and 3.3 h at 70 ºC. In the case of ultrasonically assisted drying assays at 60 W, the drying times ranges from 4.6 h at 40 ºC to 2.2 h at 70 ºC.

As can be observed, in **Figure 4.28**, not only the temperature, but also the US application affected drying kinetic. For all the temperatures tested, the use of US improved the drying rate, and the higher the ultrasonic power

applied, the faster the drying. Thus, for example, at 60 ºC, the drying time needed to reach a moisture content of 0.3 (kg W/kg DM) was reduced from 4.4 h (0 W) to 2.5 h by applying an ultrasonic power of 60 W. Average drying time reductions by US application in all assays ranged from 13 to 44%. García-Pérez et al. (2009), Ortuño et al. (2010), Ozuna et al. (2011) and Cárcel et al. (2011) reported reductions in drying time of about 30 and 32% in carrots, 53% in lemon peel, 45% in orange peel and 40% in potatoes dried at 40 ºC, with an air velocity of 1 m/s and applying ultrasonic powers of until 90 W. Even larger drying time reductions have been found for eggplant, in which applying an ultrasonic power of 90 W a drying time decrease of 72% was found (García-Pérez et al., 2011). Eggplant has a highly unconsolidated tissue with porous structure (Wu et al., 2007) and, consequently, it is more influenced by US application than other vegetables and fruits, such as strawberry.

In strawberry, an US pre-treatment during osmotic dehydration have been also tested to improve the effectiveness of the further drying. Thus, García-Noguera et al. (2010) observed a drying time reduction of about 50% in strawberry halves when the samples were previously treated with US (25 kHz) in a 50% sucrose solution (30 ºC for 30 min) prior to drying (60 ºC, 0.5 m/s and 16% air relative humidity). In the same work, the authors also tested the ultrasonic pre-treatment in distilled water, which resulted in an air-drying time reduction of 18% as compared to the untreated samples. In this case, the US effects are linked to structural changes of fruit tissue brought about by the intense cavitation produced in liquid media. Among other factors, it was reported that US increased the sucrose added to strawberry, changing, therefore, the nature of the fresh product and so, the drying behavior.

Shrinkage

Under the experimental conditions here assayed, it was assumed that the US application and air temperature did not significantly affect the shrinkage; thus the monitoring of this phenomenon was carried out during the drying of strawberries at 70 ºC in nonUS assays. As afore-mentioned in Materials and Methods section, assuming the isotropy of the material,

strawberries cubes of 8.5 mm were used in order to assess the change of the side length from the measurement of the total volume. A linear relationship was obtained between volume (V/V_0) and moisture ratios (W/W_0) (**Figure 4.29**). According to the literature, similar relationships describing the shrinkage have been reported in other vegetables and fruits (Koc et al., 2008; García-Pérez et al. 2011; Schössler et al., 2012b). Thus, the slope value (0.692) showed in **Figure 4.29** for strawberry drying was in the range previously reported by Ramallo and Mascheroni (2013) for pineapple samples dried at temperatures between 45 and 75 ºC (0.652-0.785). However, these values were lower than those found in eggplant during a conventional drying at 40 ºC and 1 m/s of air velocity (0.929-0.960) (García-Pérez et al., 2011) and with a halogen moisture analyzer at 70-90 ºC (Aversa et al., 2011). These differences might be due to the different product structure and drying method which can affect the collapse of cell matrix.

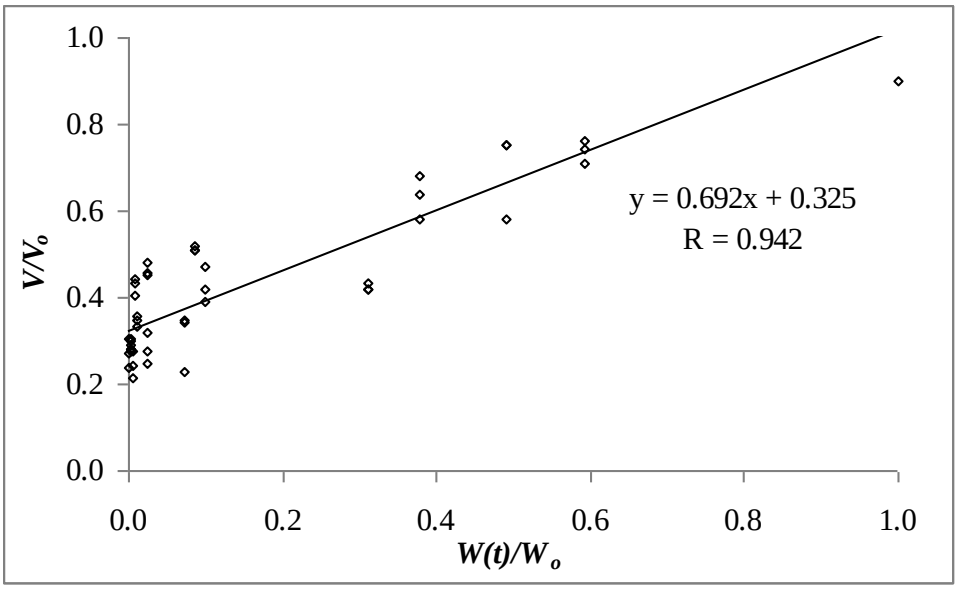

Figure 4.29 Variation of volume and moisture ratio for strawberry cubes during drying (2 m/s, 70 ºC).

From the afore-mentioned relationship, Eq. 9 was obtained to determine the change of the characteristic diffusion path length during drying.

$$L = \sqrt[3]{(0.692 \times \frac{W}{W_0} + 0.325) * L_0^3} \qquad R = 0.942 \qquad (9)$$

where subscript 0 makes reference to the initial time. This equation was included in the modeling of drying kinetics to provide a more realistic determination of the effective moisture diffusivity.

Desorption isotherms

Experimental desorption isotherm of strawberry determined at 25 ºC is shown on **Figure 4.30**. According to Brunauer's classification (Brunauer et al., 1940) it may be classified as type III "Raoult's type" (Blahovec & Yanniotis, 2010), as products with small amounts of water at low a_w and large amounts at high a_w levels (García-Pérez et al., 2008). Type III curves were also observed for other fruits (Roos, 1993; Lim et al., 1995; Mäskan & Gögüs, 1998; Vázquez et al., 1999).

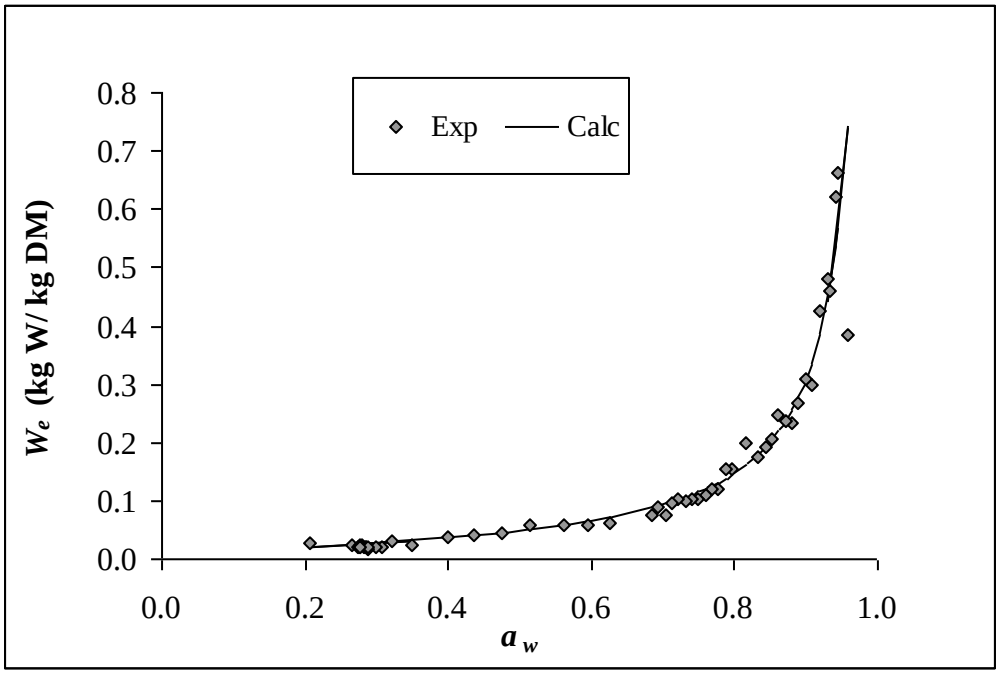

Figure 4.30 Experimental and calculated (BET model) sorption isotherm of strawberry samples at 25 ºC.

The BET parameters identified from modeling of the experimental data of a_w and moisture content in strawberry samples were 0.316 kg W/kg DM for W_m and 3.63 for C. Despite the high experimental variability observed, BET model reached a high value of VAR (96.3%) and low RME (9.4%). The adequate fit of the model to the experimental data is also shown in

Figure 4.30, thereby, it was considered an appropriate and simple model to represent the experimental data. The identified W_m value was slightly higher than those reported for cherries and blueberries by Yu et al. (1998) (0.12-0.13 kg W/kg DM) and Vega-Gálvez et al. (2009) (0.08-0.13 kg W/kg DM), but lower (0.74-0.95 kg W/kg DM) than others reported for strawberries (Moraga et al., 2004). In the case of C value, Garau et al. (2006), García-Pérez et al. (2008) and Molina Filho et al. (2011) obtained similar figures for orange peel (2.4), lemon peel (1.4-4.9) and pumpkins (1.9-4.2), respectively. Since parameter *C* is related to water molecules-food matrix interactions (Erbas et al., 2005), results obtained for strawberry could be associated with relative low heat of sorption, compared with values reported by Vega-Gálvez et al. (2009) for blueberry(*C*: 101.45 at 40 ºC). In some of the previous cited works (Moraga et al., 2004; Garau et al., 2006; García-Pérez et al., 2008), GAB equations were simplified to BET models due to *K* constant of GAB model was nearly equal to one, thus, they were used for comparison.

The BET model here proposed will be used in the following section for modeling of drying kinetics; it will contribute to quantify the convective water flux in the interface (Eq. 5).

Influence of power ultrasound on mass transport

Table 4.37 shows the average effective moisture diffusivities (D_e) and the mass transfer coefficients (k) identified from the fitting of the diffusion model for slab geometry. The diffusion model, considering ER to mass transfer and a significant shrinkage, provided an adequate description of experimental data. Independently of the drying conditions, the VAR values obtained were above 99% and, in general, the MRE were under 5%. This fact involves that the mass transfer phenomena at these experimental conditions was adequately described by the diffusion model used for strawberries slabs drying.

Table 4.37 Modeling of drying kinetics of ultrasonically assisted drying of strawberry. Identified parameters and statistical analysis. Means ± SD

Experiences	D_e $(10^{-10} m^2/s)$	k $(10^{-5} kg\ W/m^2/s)$	*VAR (%)*	*MRE (%)*
nonUS-40	0.763 ± 0.075^{a}	1.446 ± 0.256^{a}	99.74	3.79
US-40-30	0.898 ± 0.173^{ab}	1.380 ± 0.405^{a}	99.98	3.30
US-40-60	1.117 ± 0.086^{bc}	1.633 ± 0.160^{ab}	99.97	3.26
nonUS-50	0.947 ± 0.089^{ab}	1.630 ± 0.194^{ab}	99.97	4.25
US-50-30	1.267 ± 0.154^{cd}	1.750 ± 0.311^{abc}	99.90	4.23
US-50-60	1.500 ± 0.099^{de}	2.017 ± 0.180^{bcd}	99.97	3.84
nonUS-60	1.305 ± 0.123^{cd}	2.008 ± 0.234^{bcd}	99.85	4.09
US-60-30	1.470 ± 0.091^{def}	2.380 ± 0.150^{cde}	99.99	1.06
US-60-60	1.737 ± 0.089^{efg}	2.593 ± 0.170^{de}	99.98	2.98
nonUS-70	1.772 ± 0.112^{fg}	2.868 ± 0.202^{ef}	99.89	6.75
US-70-30	1.937 ± 0.101^{g}	3.277 ± 0.509^{f}	99.93	4.67
US-70-60	2.293 ± 0.110^{h}	3.387 ± 0.320^{f}	99.88	3.55

[1]Means with the same superscript letter (a-f) within the same column showed no statistically significant differences for their mean values at the 95% confidence level (LSD).

Figure 4.31 also illustrates how the model fits to the experimental data taking as example the assays carried out at 70 ºC and 60 W. It is observed that the model provides a good description of the drying kinetic due to the closeness between experimental and calculated values.

D_e values (from 0.763 to 2.293 x 10^{-10} m^2/s) were within the ranges previously reported for convective drying of strawberries and also in the common range for foodstuffs (between 10^{-11} and 10^{-9}) (Doymaz, 2008b). Furthermore, D_e values were close to those identified by García-Pérez et al. (2009, 2012); Ozuna et al. (2011) and Cárcel et al. (2011) in US assisted drying of several fruits and vegetables.

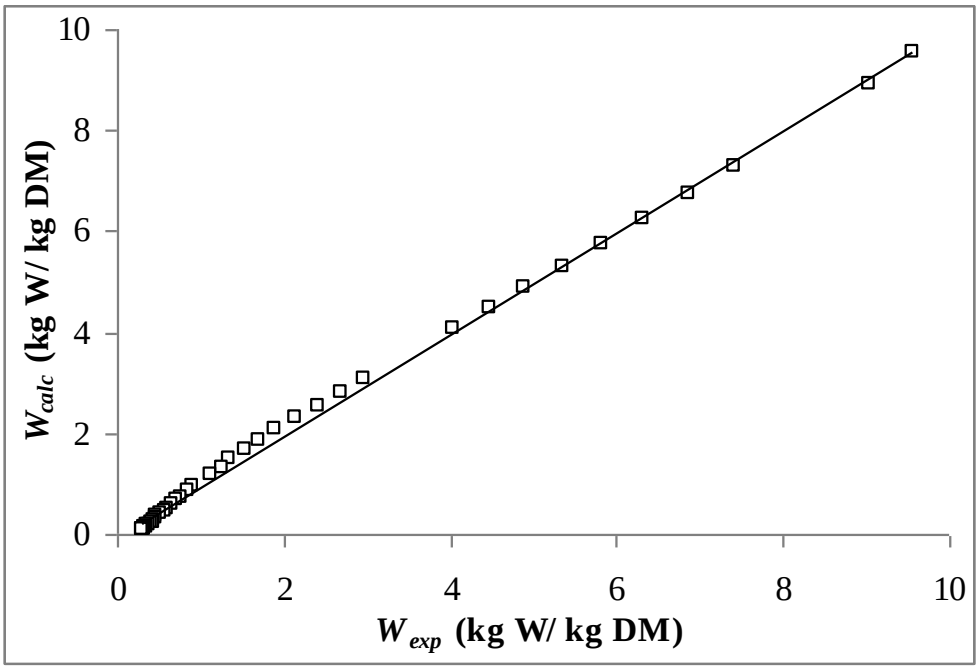

Figure 4.31 Experimental (W_{exp}) *vs.* calculated (W_{calc}) moisture content of strawberry slabs dried at 70 ºC applying an ultrasonic power of 60 W (US-70-60 drying experiment).

Effect of temperature

As can be observed in **Table 4.37** for every ultrasonic power level tested, the increase of the drying temperatures increased the D_e and k parameters identified. From 40 ºC to 70 ºC, D_e and k value were duplicated in both nonUS and US experiments. Similar results were obtained by Doymaz (2008b) for convective drying of strawberry between 50 and 65 ºC (D_e: 4.95 x 10^{-10} m^2/s and 1.09 x 10^{-9} m^2/s, respectively). D_e values reported by Doymaz (2008b) were identified using a diffusion model without considering ER, thus, in certain way, they also include the effect of temperature over external mass transport coefficients. Therefore, their direct comparison with the results here reported is complicated. Taking into account models considering ER to mass transfer, larger improvements of D_e have been identified. Thus, García-Pérez et al. (2006b), for carrots cubes drying, reported a D_e increase of 137% when temperature increased from 40 ºC (1.93 x 10^{-10} m^2/s) to 70 ºC (4.57 x 10^{-10} m^2/s). The temperature increase activates water molecules, speeding up the water transfer in the particle. Although, the magnitude of final effect observed in the D_e is dependent on

the product properties, affecting structural parameters, such as porosity or tortuosity. Therefore, the temperature effect on D_e is largely product's dependent.

The influence of temperature on D_e was quantified from an Arrhenius type relationship (Simal et al., 2005; Sablani & Rahman, 2008; Vega-Gálvez et al., 2008) (**Figure 4.32**).

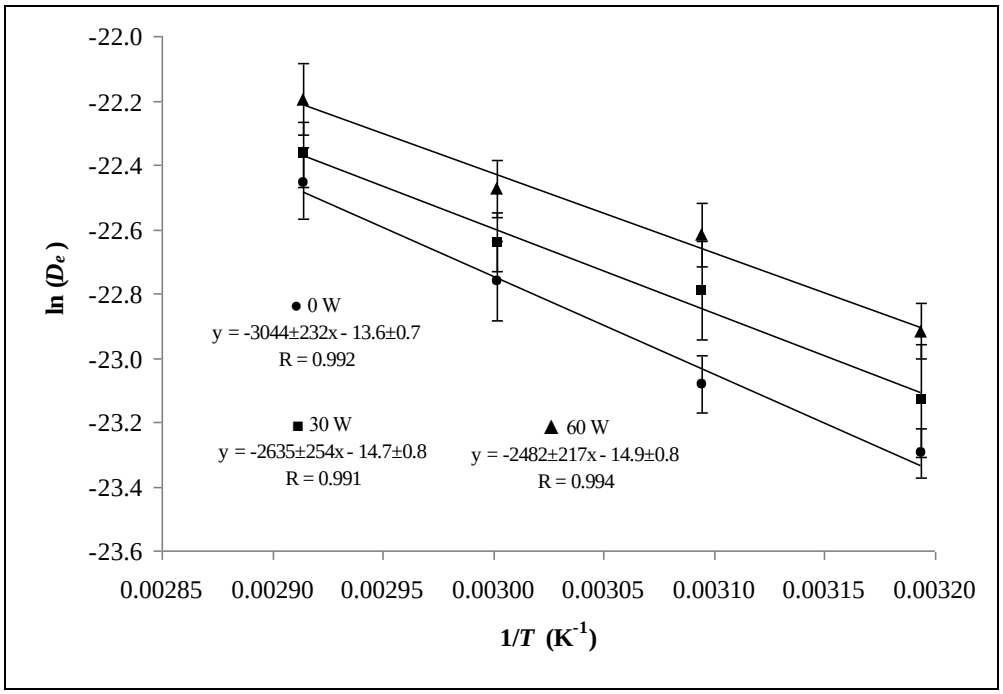

Figure 4.32 Fit of Arrhenius equation (continuous line) to the identified D_e for strawberry drying assisted by power ultrasound. Experiments carried out at 2 m/s applying different ultrasonic powers (0, 30 and 60 W).

Linear correlation coefficients higher than 0.99 were found for the different assays carried out (0, 30 and 60 W). The E_a value calculated (25.3 ± 1.9 kJ/mol) in nonUS experiments (0 W) was within the range of those proposed for other products: 28.4 kJ/mol for carrots (Doymaz, 2004b); 21.9 and 32.3 kJ/mol for kiwi (Simal et al., 2005), 18.1- 43.2 kJ/mol for apple (Vega-Gálvez et al., 2008), 42.3 kJ/mol for peas (Senadeera et al., 2003) and 28.6-31.5 kJ/mol for papaya (Vega & Lemus, 2006). E_a values identified decreased to 21.9 ± 2.1 and 20.6 ± 1.8 kJ/mol for assays carried out applying 30 and 60 W respectively, this being observed in the slopes of the linear relationships depicted in **Figure 4.32**. Thereby, E_a values for nonUS and 60 W experiments were significantly different ($p<0.05$).

Therefore, in certain way, the ultrasonic power affected the US application in strawberry drying, being this fact addressed in the following section.

Effect of power ultrasound

The US application increased the identified D_e and k values for all the temperatures tested (**Table 4.37**), being the effect dependent on the power applied, so, the higher the power, the larger the improvement. In the case of D_e, the average increase was of 18 and 42% for 30 and 60 W, respectively. Similar improvements were reported in the literature for the US assisted drying of other fruits and vegetables. Thus, in potatoes, D_e values increased by 19% (30 W) and 41% (60 W), when drying at 40 ºC (Ozuna et al., 2011). In carrots by applying 60 W, only an increase of 17% was obtained (40 ºC); whereas average improvements of 62% and 100% at 30 W and 60 W were reported in lemon peel slabs dried at 40 ºC (García-Pérez et al., 2009). In other more porous materials such as eggplant, García-Pérez et al. (2011) reported improvements up to 92 and 211%, by applying US at 30 and 60 W, respectively. As reported in the literature (Riera et al., 2011), the alternating expansions and contractions cycles produced by power US in the materials should be the main phenomenon speeding-up the inner water movement, being this manifested in the increase of D_e.

As already mentioned, the air temperature during drying affected the US application. Thus, the average improvement of D_e at low temperatures (40, 50 ºC) was of 25.8 and 52.3% at 30 and 60 W, respectively, being this reduced to 11.1 and 31.5% at high temperatures (mean for 60 and 70 ºC, respectively). Likewise, the negative effect of high temperatures on the US application has been also reported in the drying of carrot cubes (García-Pérez et al., 2006b). This phenomenon is linked to the fact that at high temperatures, and due to the large thermal energy available in the medium, the energy ratio provided by US over the total energy could be almost negligible (Riera et al., 2011).

Power US application also affected the convective water transport. As is illustrated in **Table 4.37**, at any temperature assayed, k value increased significantly ($p<0.05$) by US application. The average k increase compared to nonUS experiments were 13% and 50% at 30 W and 60 W respectively. US

waves creates turbulences, oscillating velocities and microstreaming in the interfaces, which leads to a reduction of the boundary layer thickness and so, to an increase in the k values (Puig et al., 2012). Previous works have confirmed the ability of US not only to improve the mass transfer in the interface but also to provoke structural changes on the product surface (Ortuño et al., 2010; Cárcel et al., 2011; Ozuna et al., 2011). From microstructural observations, during orange peel drying, Ortuño et al. (2010) observed the spread of waxy compounds on cuticle surface, which was linked to the great turbulence generated by US in the interface.

Conclusions

The results here reported highlight that ultrasonic application during convective drying of strawberries is a promising supporting alternative due to its ability to improve mass transport phenomena and so, reducing drying time. The effect of power US on the strawberry drying was dependent on the power and the temperature applied. Thus, the higher the ultrasonic power applied, the faster the drying, while, the higher the drying temperature, the less intense the ultrasonic effect. From modeling, a significant effect of US application on both the D_e and the external k was pointed out. Further work will be needed to elucidate how US influences on the quality of the material being dried.

4.3.1.2.2 Impacto de los ultrasonidos de potencia sobre la calidad de fresas secadas por convección

Impact of power ultrasound application on the quality of convective dried strawberries

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Journal of Agricultural and Food Chemistry (en redacción)

Abstract

In this paper, a study on the quality of strawberries dehydrated in a convective system assisted by power ultrasound at 40-70 °C and 30 and 60 W has been carried out. For comparative purposes, treatments under the same temperatures but without ultrasound application were taken as controls. The effect on vitamin C retention, initial steps of Maillard reaction and rehydration properties has been evaluated. High levels of retention of vitamin C were observed in all cases (65-84%), ultrasound treated samples showing lower values as compared to samples dried without ultrasound. The amounts of 2-furoylmethyl amino acids, as indicators of initial steps of Maillard reaction, were also lower in the former. In general, the quality of ultrasound treated samples was higher than that of commercial samples reported in the literature. In addition, the storage at room temperature of strawberries treated at 70 °C with and without ultrasound pointed out the microbiological stability of samples during at least 6 months and a similar nutritive quality evolution regardless the ultrasound application. According to the obtained results, the application of ultrasound during convective drying is an effective system to obtain high quality dried strawberries.

Introduction

A great awareness of healthy eating habits can be noticed among developed country consumers and public and private institutions (Giampieri et al., 2012). In this sense, the interest in fruits and vegetables has

increased during the last decades because of their considerable content in health promoting compounds (Basu et al., 2010). Among fruits, strawberry (*Fragaria x ananassa*) highlights not only by its nutritive value and palatability but also as a relevant source of bioactive compounds (Proteggente et al., 2002), vitamin C being one of the most important. Specific studies on vitamin C related to health benefits include their role in prevention of inflammation, oxidative stress, cardiovascular disease, cancer, type 2 diabetes, obesity and neurodegeneration (Du et al., 2009; Giampieri et al., 2012).

Fresh fruits and, particularly, strawberry are highly perishable, and processing is an alternative to extend their shelf-life. Among the available processes, dehydration and, mainly convective drying, is a common option to obtain products with reduced moisture content and easy to store and transport. Other advantages are linked to the diversification of the products than can be offered to consumers, since they can be directly intake or used as ingredients. Thus, dehydrated fruits have a huge amount of applications in breakfast cereals, bakery, desserts and confectionary products.

However, during processing and storage of dehydrated fruits, numerous physical and chemical changes can negatively affect their nutritional and sensorial quality (Di Scala & Crapiste, 2008; Derossi et al., 2010). One of the most important physical modifications is the shrinkage due to cellular structure stress brought about by high drying temperatures and long drying times, which can affect the rehydration properties (Mayor & Sereno, 2004; Frías et al., 2010a; García-Pérez et al., 2011).

Regarding chemical reactions, vitamin C degradation is perhaps the most important change that can take place during fruit drying (Santos & Silva, 2008). In the case of strawberries, several works have been focused on the loss of vitamin C during drying, and different retention values, between 98 and 16%, have been reported depending on the type and severity of the treatment (Asami et al., 2003; Böhm et al., 2006; Wojdylo et al., 2009). Other reactions that can increase quality losses of dried vegetables and fruits involve essential amino acids (Keutgen and Pawelzik, 2008), Maillard reaction (MR) being one of the most relevant. This reaction takes place between the free amino groups of amino acids, peptides and proteins, and reducing carbohydrates and it is favored at low water activities and high temperature

conditions (Corzo-Martínez et al., 2012). 2-FM-AA, obtained by acid hydrolysis of Amadori compounds formed during the initial steps of this reaction, are recognized as sensitive indicators of the early detection of changes in the nutritional and sensorial quality of dehydrated commercial fruits (Sanz et al., 2001).

On the other hand, during recent years, emergent technologies have been proposed to reduce the problems related to conventional drying techniques. As a non-thermal strategy in drying of fruits and vegetables, the application of high power ultrasound (US) represents a promising alternative. During drying, US produces in solid media alternative compressions and expansions cycles, namely "sponge effect", and the creation of internal microchannels that facilitate the water removal (Cárcel et al., 2012). Moreover, US generates microstirring at the solid-fluid interfaces that makes easier the mass transfer. In general, it has been reported that the application of US during drying of fruits and vegetables affects the kinetic of dehydration, decreasing significantly the processing time (Ortuño et al., 2010; Ozuna et al., 2011; Puig et al., 2012). However, scarce data are available in the literature on the final quality of fruits and vegetables dried under the influence of US. Soria et al. (2010) reported that dried carrots in a US system by direct contact presented, in general, similar quality (total polyphenols, rehydration ratio, protein profile) to freeze-dried samples. Moreover, the advance of the MR was much slower as compared to commercial dried carrots. These US treated samples had significantly lower losses of vitamins than conventionally dried carrots (Frías et al., 2010b). Very recently, our research group has studied the kinetic of moisture loss in strawberry samples dried in a convective system assisted by US (40-70 °C; 0-60 W) and, a significant increase in the effective moisture diffusivity and the mass transfer coefficient was found (Gamboa-Santos et al., in writing); however, no evaluation of the overall quality was carried out. Thus, the aim of this work was to investigate the effect of the application US during the drying of strawberries (0-60 W) on vitamin C retention, 2-FM-AA formation, rehydration properties, and the microbial stability and nutritional quality during storage.

Materials and Methods

Ultrasound assisted drying treatments

Fresh strawberries (*Fragaria x ananassa* Duch) were purchased from a local market in Valencia (Spain) and stored at 5 °C for up 3 days until processing. Prior to drying, samples were washed in tap water to remove external impurities and cut into 2.5 ± 0.5 mm thickness slabs. Drying of strawberries was carried out in a US assisted convective drier prototype previously described by García-Pérez et al. (2006b). The processing conditions were: temperatures of 40, 50, 60 and 70 °C, electric power applied to the air-borne US transducer 0, 30 and 60 W and air velocity 2 m s^{-1}. Dried strawberry samples were coded as shown in **Table 4.38**. Slabs were placed separately in a metallic frame to allow, once introduced in the vibrating chamber, free air flow around each piece of strawberry. The initial mass of samples was 73.5 ± 3.5 g (initial moisture content 9.55 ± 0.27 kg H_2O kg^{-1} dry matter (DM)), which were dried until constant weight of 8.1 ± 1.0 g (final moisture content of 0.16 ± 0.10 kg H_2O kg^{-1} DM), for which drying times ranged from 2.4 to 5.5 h. The total weight of strawberries was recorded at 3 min intervals, during the whole drying process. Drying tests were carried out in triplicate (Gamboa-Santos et al., in writing) for the different experimental conditions tested.

Table 4.38 Codification of processing conditions applied in US assisted drying of strawberries. In brackets, processing time (hs) for each drying experiment

Power (W)	Temperature (°C)			
	40	50	60	70
0	nonUS-40 (5.3)	nonUS-50 (4.7)	nonUS-60 (4.4)	nonUS-70 (3.3)
30	US-40-30 (5.5)	US-50-30 (3.9)	US-60-30 (3.5)	US-70-30 (2.8)
60	US-40-60 (4.8)	US-50-60 (3.3)	US-60-60 (2.8)	US-70-60 (2.4)

Samples characterization

Dry matter (DM) content was determined gravimetrically (AOAC, 1990a). Kjeldahl method was performed to determine total nitrogen (TN),

using 6.25 as conversion factor (TN x 6.25) to calculate the protein content (AOAC, 1990b). All determinations were carried out, at least, in triplicate.

Microbiological analysis

In order to evaluate the microbiological quality, samples of strawberry dried at 40 °C (nonUS-40; US-40-60) and 70 °C (nonUS-70; US-70-60) were analyzed after processing and during their storage at ambient temperature for the total aerobic, enterobacteria, molds and yeasts and sporulated aerobic and anaerobic microorganisms.

Samples (1.5 g) were placed with 27 mL of peptone water (sterile peptone, 2.55%) in a sterile stomacher bag. The samples were then homogenized into the stomacher for 1 min (230 rpm), filtered and then diluted with peptone water for the microbial count. Serial dilutions were performed in triplicate. The total aerobic bacteria and enterobacteria counts were determined by plating appropriately diluted samples onto plate count agar and violet red bile dextrose agar, respectively. The samples were incubated at 30 ± 1 °C for 72 h for total aerobic bacteria and for 24 h for enterobacteria. Yeasts and molds were plated on sulphite cycloserine agar and incubated at 25 ± 1 °C for 5 days. For aerobic and anaerobic sporulated counts, brain heart infusion agar was used; incubation was carried out at 37 ± 1 °C for 48 h. All microbial counts were reported as logarithm of colony forming units per gram (log CFU g^{-1}). Microbial quality was evaluated according to the European legislation for vegetables and fruits (EC 2073/2005). All culture media were of Difco (Difco Co., Detroit, MI, USA)

Determination of vitamin C

Strawberry extracts were prepared in triplicate by adding 12.5 mL of 0.4% oxalic acid to 0.25 g of strawberry samples and homogenizing for 1 min at 13,500 rpm using an Ultra-Turrax T-35 homogenizer (IKA Labortechnik, Janke & Kunkel, Saufen, Germany) (Gamboa-Santos et al., 2013b). After addition of 2.5 mL of a 5 mg mL^{-1} solution of D,L-dithiothreitol, to reduce the dehydroascorbic acid to ascorbic acid, strawberry extracts were kept at room temperature in the darkness for 30 min. Prior to centrifugation at 3,200*g* for

5 min, slurries were made up to 25 mL with Milli-Q water. The supernatant was filtered through 0.45 µm syringe filters prior to their determination by HPLC.

Total vitamin C content of strawberries was determined by liquid chromatography with diode array detector (RP-HPLC-DAD) on an Agilent Technologies 1220 Infinity LC System – 1260 DAD (Boeblingen, Germany). Vitamin C separation was done with an ACE 5 C_{18} column (ACE®, UK) (250 mm length, length x 4.6 mm internal diameter, 5 µm), at 25 °C, using 5 mM KH_2PO_4 buffer (pH 3.0) as the mobile phase. The elution program was performed under isocratic conditions at a flow rate of 1 mL min^{-1} for 10 min. Automatic injection volume was 20 µL and for data acquisition and processing, the Agilent ChemStation software was used (Agilent Technologies, Germany).

The external standard method was used to quantify the vitamin C content, using a commercial standard of ascorbic acid (Sigma) (0.3-50 mg L^{-1}). Results were expressed as mg of total vitamin C 100 g^{-1} DM and the percentage of retention was calculated taking into account the initial content of vitamin C in the raw strawberries.

Analysis of 2-furoylmethyl-amino acids

2-FM-AA were determined by ion-pair Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) (Soria et al., 2010) with a C_8 column (250 mm length x 4.6 mm internal diameter, Alltech, Lexington, KY) at 37 °C. Phase A (4 mL L^{-1} acetic acid) and phase B (3 g L^{-1} KCl in phase A solution) were used to make a binary gradient with the following elution program: 0-12.0 min, 100% A; 20.0–22.5 min, 50% A and 50% B; 24.5-30.0 min, 100% A. The flow rate was 1.2 mL min^{-1}, the injection volume was 50 µL and detection was done at 280 nm (LCD Analytical SM 4000 detector).

Hydrolyzed samples were prepared with 0.25 g of dried strawberries and 4 mL of HCl 8 M. Samples were placed in an oven at 110 °C, for 23 h, under inert atmosphere (nitrogen) using a Pyrex screw-cap vial with polytetrafluoroethylene-faced septa. After filtering (paper filter Whatman no. 40), 0.5 mL of the resulting hydrolyzed were passed through a Sep-Pack C_{18}

cartridge (Millipore, MA) activated with 5 mL of methanol and 10 mL of Milli-Q water. The filtrate was then eluted with 3 mL of 3 M HCl.

Quantification of samples was performed by the external standard method, using a commercial standard of furosine (Neosystem Laboratoire, Strasbourg, France). Values were expressed as mg 100 g^{-1} protein and analyses were done at least in triplicate.

Rehydration ability

Dried strawberry samples were rehydrated by immersion in distilled water (solid-to-liquid ratio 1:50) at 20 °C for 2 h, according to Soria et al. (2010). Prior to weight the samples, strawberry slabs were placed onto paper towels to remove the superficial water. Each rehydration experiment was performed in triplicate and rehydration ratio (RR) was calculated as follow:

$$RR = m_r/m_d,$$

where m_r and m_d represent the weight of the rehydrated and dehydrated strawberry, respectively.

Rehydration water was placed in a preweighted vial and dried in a conventional oven for 24 h at 102 °C. The residue was weight to determine the percentage of solids leaching losses (LL, % dry basis) with respect to the initial weight.

Storage assays

Strawberry dried samples at 70 °C without US (nonUS-70) and with US (US-70-60), were packed in polypropylene individual bags under vacuum, and, then, were stored in the dark for a period of 6 months at 25 ± 1 °C under controlled conditions.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) (Fischer LSD Test, $p < 0.05$) by applying the Statgraphic 5.1 statistical package (Statistical Graphics Corp., Rockville, MD).

Results and discussion

Microbiological quality

The microorganism determination of studied samples (nonUS-40, US-40-60, nonUS-70 and US-70-60) after processing, indicated that the microbial load (total aerobic bacteria, enterobacteria, yeast and molds, aerobic and anaerobic sporulated counts) was, in all cases, lower than 3 log CFU g^{-1}. In addition, the storage of nonUS-70 and US-70-60 samples for 6 months at room temperature did not significantly ($p < 0.05$) increase the counts of microorganisms. According to the microbiological criteria recommended for vegetables, fruits and derivatives, the maximal limits are 2-3 log CFU g^{-1} for molds and yeasts and 5 log CFU g^{-1} for aerobic mesophiles (EC 2073/2005). Therefore, processing and storage conditions were adequate to guaranty the microbial stability of samples during, at least, 6 months.

Retention of vitamin C

As aforementioned, the nutritional quality of dried strawberry samples was evaluated from their vitamin C content. In agreement with other works on the variations of nutritional quality during food processing, vitamin C is considered a compound very sensitive to processing conditions, non-subjective and relatively easy-to-measure criterion of food quality (Ryley, 1989). It has been found that if vitamin C is conveniently retained, other nutrients can be also well preserved (Shitanda and Wanjala, 2006).

The vitamin C amount of the raw strawberry samples was in the range 271.9-494.0 mg 100 g^{-1} DM, being this variability probably linked to intrinsic factors such as cultivar, growth conditions and degree of maturity (Mcminn & Magee, 1997). Wojdylo et al. (2009) reported contents of vitamin C in the fresh fruits ranging from 340.2 to 680.2 mg 100 g^{-1} DM.

Figure 4.33 illustrates the vitamin C retention (taking into account the initial content in the raw fruit) obtained for strawberries dried at temperatures between 40 and 70 °C, with and without US application (30 and 60 W). As can be observed, high levels of retention (65-84%) were found in all processed strawberry samples. Considering only the effect of temperature, in general terms, the highest values of retention were found after treatments

carried out at 40 and 50 °C, being the vitamin C degradation higher when the temperature increased. Thus, at 60 and 70 °C a noticeable reduction in the retention of this vitamin was observed. No significant differences were detected between both temperatures, probably due to the fact that the process carried out at 70 °C was shorter than those done at 60 °C (**Table 4.38**). Values of 70-81% and 40-74% were obtained in a previous work where strawberry samples were dried at 60 °C (4 m s^{-1}) and 70 °C (2 m s^{-1}), respectively, for 3-7 h in a convective drier (Gamboa-Santos et al., submitted). Wojdylo et al. (2009) found retention values of ascorbic acid of 30% for strawberry samples (var. *Kent* and *Elsanta*) dehydrated at 70 °C, 1 m s^{-1}, for 9 h. Böhm et al. (2006) reported retentions of 31-42% for ascorbic acid in three strawberry varieties dehydrated at 60 °C, 5 m s^{-1} during 220 min.

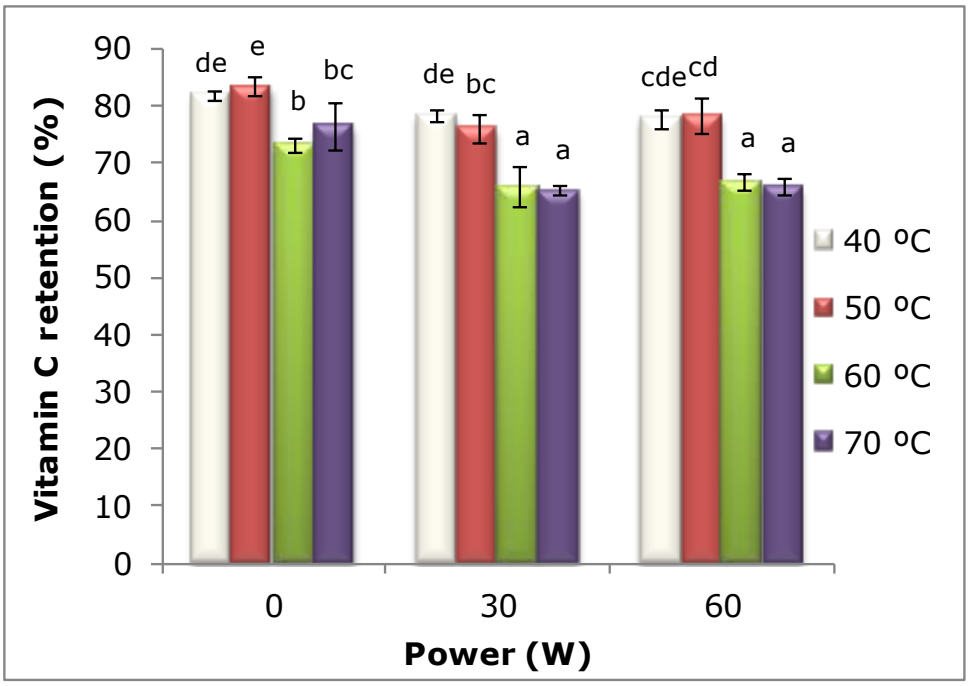

Figure 4.33 Effect of power US application and temperature of drying on the retention of vitamin C in dried strawberries (values referred to the initial content in the raw fruit).

Regarding the influence of US application, a significant decrease of vitamin C retention was found when US was applied at high temperatures, thus, this effect being evident at 60 and 70 °C (**Figure 4.33)**. However, at 40 and 50 °C, US dried samples presented percentages of vitamin C retention similar to samples dried without US application, this fact not being dependent

on the power applied (30 or 60 W). In spite of the fact that the treatments at high temperatures (60 and 70 °C) and US were shorter as compared to those without US application (**Table 4.38**), more degradation of vitamin C was observed in the former, most likely due to a combined effect between both factors, temperature and US. According to Dennison et al. (1978), US could facilitate the air penetration in the sample and, as it is known, the oxygen presence is one of the most important factors in the stability of ascorbic acid. It is noteworthy that, even under the most severe conditions (70 °C and 60 W), the retention values of vitamin C were high (65%) and within the range previously reported in the literature for convective dried strawberry samples, as aforementioned. Moreover, the final content of vitamin C in these strawberry samples (247.6 mg 100 g^{-1} DM) were higher than those of commercial products analyzed in our laboratory (64.7 mg 100 g^{-1} DM) (Megías-Pérez et al., 2011).

To date, no previous studies have been performed on the impact of US application on vitamin C degradation during convective drying of fruit. Frías et al. (2010b) observed retention values of vitamin C ranging from 82-92% in sliced carrots (4 x 24 mm) subjected to dehydration in an US system by contact (100 W) at temperatures of 20-60 °C and drying times of 75-120 min. The higher retention values reported in that paper can be ascribed not only to the different products being dried, but also to the different drying systems used. Since, in the US system by contact, processing conditions (temperature and time) were milder than those of the air-borne US system used in our work.

Regarding the vitamin C content evolution during sample storage at 25 °C for 6 months, dried strawberry samples at 70 °C without (nonUS-70) and with (US-70-60) US application were 78.6 and 85.5 mg 100 g^{-1} DM, that meant a loss of 57.1 ± 0.9% and 57.0 ± 2.6% of vitamin C, respectively compared to just dried samples. These values of vitamin C degradation during the storage are close to those reported in the literature. In dried fruits, few studies have been addressed on the vitamin C losses during the storage period. Del Caro et al. (2004) investigated the ascorbic acid losses of two varieties of prunes subjected to drying at 60-85 °C and stored at 20 °C. They found ascorbic acid retentions ranging from 50 to 69% after 4-8 months of storage. In dried vegetables, Peñas et al. (2012) reported highly variable

(28 at 93%) reductions in vitamin C content of vacuum packaged garlic, onion, potato and carrot commercial and freeze-dried obtained in laboratory samples after 12 months of storage at room temperature. Kim et al. (2006) in dried pepper (70 °C during 6 h) reported a loss of 75% of vitamin C during 6 months of storage at 20 °C. Losses of vitamin C close to 60% were also observed by Megías-Pérez et al. (2012) during the storage of crunchy pepper obtained by texturization by expanded microperforation and packed in plastic bags with modified atmosphere.

Assessment of initial steps of Maillard reaction

Figure 4.34 depicts the RP-HPLC chromatographic profile of 2-FM-AA obtained after the acid hydrolyzate of dehydrated strawberry samples at 70 °C and 60 W (US-70-60). Identification of 2-FM-AA of γ-aminobutiric acid (2-FM-GABA, peak 1) and of lysine plus arginine (2-FM-Lys + 2-FM-Arg, peak 2) were tentatively carried out by comparing the retention times with data obtained for standards synthesized in our laboratory and by coinjection with these standards (Sanz et al. 2001; Soria et al. 2010). The composition of free amino acids was also taken into account (Blanch et al., 2012).

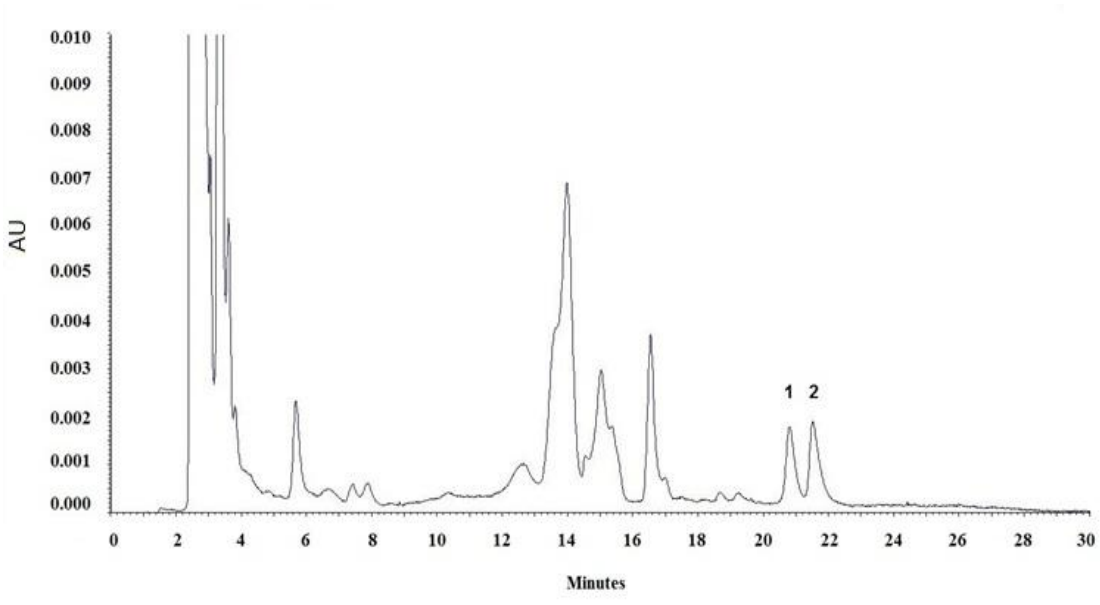

Figure 4.34 RP-HPLC-UV chromatogram of 2-FM-AA in acid hydrolyzates of US assisted convective dried strawberries (US-70-60). (1) 2-FM-GABA, (2) 2-FM-Lys + 2-FM-Arg.

Figure 4.35 shows the content of 2-FM-AA found in strawberry samples dried at the different processing conditions here assayed. As observed, 2-FM-Lys + 2-FM-Arg (**Figure 4.35a**) were formed in higher amount (up to 90 mg 100 g^{-1} protein) as compared to 2-FM-GABA (**Figure 4.35b**) (up to 60 mg 100 g^{-1} protein), due to the different reactivity of the corresponding amino acids (Wellner et al., 2011).

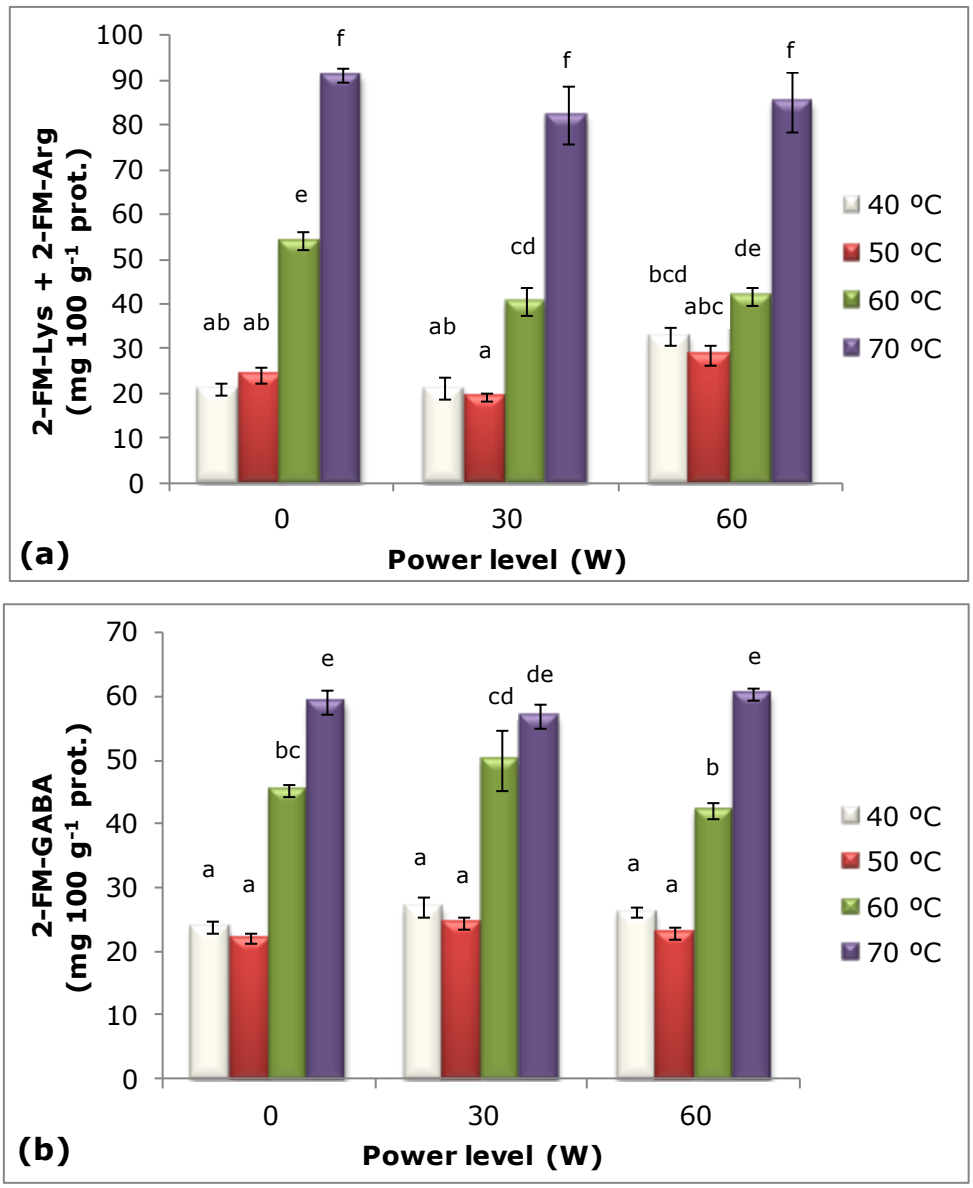

Figure 4.35 Effect of power US application and temperature of drying on the 2-FM-Lys + 2-FM-Arg (a) and 2-FM-GABA (b) content of dried strawberries.

Considering only the effect of temperature, above 50 °C 2-FM-AA contents increased with the temperature, being this effect particularly evident at 70 °C. Gamboa-Santos et al. (submitted) found 2-FM-Lys + 2-FM-Arg and

2-FM-GABA amounts in the ranges 35-265 mg 100 g^{-1} protein and 30-198 mg 100 g^{-1} protein, respectively, in strawberry samples dried by convection (in a convective drier) at 40-70 °C, air flow rates of 2-8 m s^{-1} and 3 h, whereas no formation of these compounds was detected during the first h of treatment. Moreover, in commercial dehydrated strawberry samples, Megías-Pérez et al. (2011) quantified 2-FM-Lys + 2-FM-Arg contents in the range 46-475 mg 100 g^{-1} protein for lyophilized samples and values up to 982 mg 100 g^{-1} protein for convective ones. These indicators were also previously detected in commercial samples of dried raisins, apricots, dates and figs and the concentrations were between 7.7 at 62.5 mg 100 g^{-1} product and 3.6 at 75.8 mg 100 g^{-1} product for 2-FM-Lys + 2-FM-Arg and 2-FM-GABA, respectively. These amounts were higher than those found in the strawberries dried without US here analyzed, with contents between 1.2 at 6.7 mg 100 g^{-1} product and 1.2 at 4.0 mg 100 g^{-1} product, respectively for both indicators.

Comparing US-treated samples with those without US, in general, very similar concentrations of 2-FM-Lys and 2-FM-Arg (**Figure 4.35a**) were found and no significant ($p<0.05$) differences were observed between treatments carried out at 30 and 60 W. As shown in **Figure 4.35b**, similar trends can be observed for the formation of 2-FM-GABA, although as above indicated, the sensitivity of this indicator is lower than that of 2-FM-Lys + 2-FM-Arg. To the best of our knowledge, no previous work has been reported on the MR assessment in dehydrated fruits assisted by US. Soria et al. (2010) studied the effect of temperature in 2-FM-AA formation in carrots dehydrated with US application (100 W) in a system by direct contact. These authors did not detect 2-FM-AA at drying temperatures below 40 °C and, at 60 °C, 2-FM-Lys + 2-FM-Arg levels up to 39 mg 100 g^{-1} protein were found. Comparing these results with the obtained in the present work, the differences could be ascribed, among other factors, to the milder conditions (temperature and time) used in the US system by direct contact.

Rehydration properties

Table 4.39 lists the rehydration ratio (RR) of strawberry samples dried at different temperatures with and without US application. As can be seen, RR values ranged from 4.0 to 5.2. These were lower to that obtained for a

laboratory freeze-dried (FD) sample (6.5) and similar to commercial FD strawberry samples (5.2 ± 1.2) (Megías-Pérez et al., 2011). In general, RR values obtained were in agreement with data reported for dried fruits and vegetables (El-Beltagy et al., 2007). Considering the conventionally dried samples (without US), RR decreased with the increase of temperature, although only at the highest temperature of drying (70 °C), significant ($p<0.05$) differences were found. This is consistent with the fact that fruit structure dried at high temperatures could be partially disrupted compared with the fresh product (McMinn & Magee, 1997) causing an irreversible shrinkage. Thus, sample cannot recover its initial moisture content, being this effect more aggressive when drying heat-sensitive materials, and drying may induce a crust formation on the surfaces, so that samples could reduce their water penetration. On the other hand, the open and porous structure of FD strawberry sample, that facilitates the water absorption, is probably responsible for of its high RR values (Shih et al., 2008).

Table 4.39 Rehydration ratio determined after 2 h of rehydration at 25 °C of previously dried strawberry samples (mean value ± SD)

Temperature (°C)	RR		
	0 W	30 W	60 W
40	5.2 ± 0.4^{d1}	4.7 ± 0.4^{bcd}	5.0 ± 0.5^{d}
50	4.8 ± 0.3^{cd}	4.7 ± 0.3^{bcd}	5.0 ± 0.3^{d}
60	4.7 ± 0.5^{bcd}	5.1 ± 0.2^{d}	4.0 ± 0.3^{a}
70	4.4 ± 0.1^{abc}	4.1 ± 0.8^{ab}	4.2 ± 0.2^{ab}

[1]Samples with the same superscript letter (a-d) within the same column showed no statistically significant differences for their mean values at the 95% confidence level.

Hardly any effect of US was observed on the RR values and the trend observed for both US powers tested (30 or 60 W) was similar to that detected in the case of samples without US. No significant ($p<0.05$) differences were observed in samples treated at 40 and 50 °C and the lowest values of RR, in general, were also found at 70 °C. In carrots, Soria et al. (2010) found higher values of RR in samples dried by US in a system by direct contact as compared to freeze-dried samples when carrots were previously blanched prior to US treatment. In the case of US treated samples here analysed an improved RR could have been expected probably due to the formation of microchannels, or other structural damages, in the fruit tissue. However, the mechanisms of US affecting water removal are multiple, including, among

others, cell disruption. The convective boundary layer can be affected by the pressure variations and microstirring induced by US (Mulet et al., 2003); thus, García-Pérez et al. (2012a) identified an intense spread of way compounds on the surface of orange peel flavedo, which was coupled to a high water evaporation rate. While, the inner water removal is mainly improved by the cyclic compressions and expansions produced by US (Gallego-Juárez et al., 1998), which in certain way could also affect internal structure, such as was observed in orange peel (García-Pérez et al., 2012a) and eggplant (Puig et al., 2012). According to Schössler et al. (2012a), the cell disruption induced by US is mainly produced in the outer layer and the damage originated in deeper layers is mainly attributed to structural modifications associated with the water removal. However, the US effects could be dependent on the effective ultrasonic power applied, for which comparing reported works carried out with different US systems results complicated. Moreover, the absorption of US as heat could also contribute to the evaporation of water from the inner tissue (Humphrey, 2007) and also could induce structural changes.

With respect to the corresponding leaching losses (LL) during rehydration of dried strawberry samples were found to be in the range 61.8-77.6% (dry basis). Similar LL values were observed by Megías-Pérez et al. (2011) in freeze dried (46.3-72.4%) and convective dried berry fruits (59.3-90.9%). These data highlight the convenience of consuming dried strawberries directly without rehydrating or rehydrated directly in foodstuffs.

Conclusions

Present results indicate that the application of US during convective drying is an adequate system to obtain dried strawberry samples with high nutritive value and adequate microbiological quality. Losses of lysine and arginine due to their participation in the MR were similar than those observed in conventional dried strawberries. Moreover, the amounts of 2-FM-AA were, in all cases, lower than those of commercial dried strawberry and other fruit samples. Values of vitamin C retention were very high (65%), even under the most severe conditions used (US-70-60). A combined effect of US and heat was observed since the lowest retention of vitamin C was found in US-

assisted dried samples at high temperature. The obtained values of vitamin C retention and rehydration properties in US treated strawberries were within the ranges reported in the literature for convective dried fruits and vegetables. Finally, in samples treated at 70 °C with and without US, during the storage at ambient temperature, no changes in the microbial counts were detected with respect to the initial processed samples, indicating the stability of US-treated samples during at least 6 months. Moreover, these samples showed similar evolution in the losses of vitamin C (aprox. 50%) during the storage period. This is the first study on the evaluation of quality of fruits, particularly strawberries, dehydrated by US-assisted drying. According to these results, US is a suitable example of new emerging technology environmentally friendly that accelerates the convective drying, allowing the obtention of dried strawberries with premium quality which satisfy the demands of the present consumers.

Acknowledgements

This work has been funded by Ministry of Science and Innovation of Spain (project AGL2007-63462), Fun-c-Food CSD2007-00063 Consolider-INGENIO 2010 and CYTED IBEROFUN (P109AC0302). J.G.S. also thanks CSIC and the EU for a predoctoral JAE grant.

Discusión general

5. DISCUSION GENERAL

La constante evolución de las tecnologías para el procesado de alimentos que se produce como resultado de las exigencias de los consumidores actuales hacia alimentos seguros, nutritivos y con buenas características organolépticas, ha desencadenado un esfuerzo importante en las investigaciones llevadas a cabo en este campo. Además, el interés actual hacia el consumo de alimentos saludables ha conducido a la industria alimentaria a nuevos retos en el diseño de nuevos productos e ingredientes con propiedades específicas. Para garantizar que esos constituyentes presentes en los productos ejerzan un efecto beneficioso para la salud resulta necesario avanzar, no sólo en la evaluación y autentificación del beneficio del compuesto, sino también en el conocimiento de las modificaciones que pueden tener lugar durante el procesado del alimento y su posterior conservación. En este sentido, es preciso disponer de procesos que mantengan tanto el valor nutritivo y las propiedades organolépticas como la bioactividad de los constituyentes de los alimentos. Así, como se ha demostrado en numerosos trabajos (Barati y Esfahani, 2012; Feng y col., 2012; Rastogi, 2012; Schössler y col., 2012b; García-Pérez y col., 2012a), la optimización de las tecnologías existentes y la introducción de otras emergentes puede conducir a una reducción importante en los tiempos y en las temperaturas de procesado, lo que resulta de gran relevancia para preservar los constituyentes termolábiles. Además, otro aspecto muy importante a considerar es la utilización de procesos más eficientes energéticamente y respetuosos con el medio ambiente que los convencionales. En este sentido, los US son un claro ejemplo de nueva tecnología con innumerables aplicaciones en el procesado de los alimentos. Revisiones recientes (Soria y Villamiel, 2010; Chemat y col., 2011; Cárcel y col., 2012; Chandrapala y col., 2012) han puesto de manifiesto el gran potencial de los US en el campo de la Ciencia y Tecnología de los Alimentos, siendo la deshidratación de vegetales y frutas una de sus aplicaciones más prometedoras.

Como es sabido, los alimentos de origen vegetal ocupan un lugar preeminente en la alimentación de hoy en día, siendo las frutas y vegetales deshidratados un segmento al que se le otorga un creciente interés. Con el

objetivo de diversificar el rango de alimentos que existen en el mercado y mejorar su calidad y así atender a las necesidades del consumidor actual, esta Tesis se ha propuesto la optimización de los procesos de deshidratación de productos vegetales comúnmente empleados en la industria agroalimentaria para, posteriormente, abordar el estudio sobre la aplicación de US en el proceso de deshidratación. Todo ello encaminado a profundizar en la potencialidad de esta tecnología emergente que podría soslayar en parte las desventajas de los procesos convencionales de deshidratación.

Entre los vegetales y frutas seleccionados, especial atención se ha prestado a zanahorias y fresas, por su elevado consumo y sus propiedades nutritivas y saludables, dado su gran contenido en constituyentes bioactivos (Erenturk y Erenturk, 2007; Giampieri y col., 2012). Con el fin de realizar una evaluación global y exhaustiva de la calidad y bioactividad de estos productos deshidratados, en base a las características de los procesos de deshidratación y a las modificaciones químicas y físicas que tienen lugar en las matrices vegetales, los indicadores seleccionados fueron: enzimas (POD y PME), vitaminas, compuestos de las etapas iniciales de la reacción de Maillard (2-FM-AA), carbohidratos, polifenoles, perfil proteico, capacidad de rehidratación, microestructura y propiedades sensoriales.

Inicialmente, dado que no se disponía de trabajos que abordaran la calidad global de este tipo de alimentos, fue preciso llevar a cabo un estudio prospectivo de muestras industriales, tanto recién procesadas como durante el período de vida útil y de muestras del comercio. En general, las muestras industriales de vegetales mostraron poseer una adecuada calidad teniendo en cuenta el contenido en humedad, carbohidratos, polifenoles y propiedades de rehidratación, ya que apenas se encontraron variaciones a lo largo del período de almacenamiento en las condiciones habitualmente empleadas en el mercado. Con respecto a los indicadores de las etapas iniciales de la RM, sólo se vieron ligeras pérdidas de lisina y arginina por su participación en dicha reacción en las muestras de zanahoria (Apartado 4.1.1.1.1). Otros indicadores tales como el contenido en vitaminas se ha visto que puede disminuir gradualmente durante el almacenamiento bajo dichas condiciones, tal como lo habían reseñado otros autores (Peñas y col., 2012).

En el estudio sobre la calidad de frutas deshidratadas comerciales comunes y tropicales (Apartado 4.1.1.1.2), se encontró, de acuerdo a los

indicadores de calidad seleccionados, que la mayor parte de muestras analizadas presentaban escasa calidad nutricional, debido a pérdidas de lisina por su participación en la RM y de vitamina C. Esto, unido al elevado contenido en azúcares de las muestras presuntamente sometidas a un previo tratamiento por deshidratación osmótica, puso de manifiesto las grandes diferencias existentes en cuanto a la composición respecto a las frutas de partida. Así, dichos productos, tal y como se procesan en la actualidad, constituyen una fuente elevada de energía pero con escaso valor nutritivo.

Una vez conocida la calidad de los vegetales y frutas deshidratados ofertados en el mercado se estudió el modo de mejorar los procesos de secado (Apartado 4.1.1.2).

Como se ha indicado, la obtención de productos vegetales con óptimas características precisa de un control exhaustivo de los procesos de deshidratación para evitar un excesivo deterioro del alimento. El conocimiento de los factores relacionados con el proceso debe ser eficientemente empleado para obtener vegetales deshidratados con sus atributos de calidad y bioactividad preservados, en la medida de lo posible. La modelización matemática es una herramienta que ayuda eficazmente en el diseño, el desarrollo y la optimización del proceso de deshidratación. A partir de elaborados estudios sobre las cinéticas de secado se pueden describir los mecanismos de la transferencia de materia y la influencia que ciertas variables del proceso ejercen sobre la misma (Mulet y col. 1989; Gekas y Lamberg, 1991; Doymaz, 2004b). Por ello, en el desarrollo de la presente Memoria, se planteó la optimización de un proceso de deshidratación en un prototipo de secado por convección (Apartado 4.1.1.2.1). En este estudio, llevado a cabo en zanahoria, se realizó un diseño experimental para encontrar las condiciones de proceso (temperatura y velocidad del aire de secado) más favorables, teniendo en cuenta parámetros derivados de la cinética de la pérdida de humedad y de la calidad del producto final, correlacionándose todos ellos con gran precisión mediante un análisis por RSM (Response Surface Methodology). La mayor parte de los tratamientos condujeron a valores de humedad seguros para mantener la viabilidad del producto final a lo largo del período de vida útil (Belitz y col., 2009a). Para el estudio de la cinética de pérdida de humedad se tuvieron en cuenta los valores experimentales de encogimiento de las muestras, lo cual permitió

identificar un primer período de secado con velocidad constante cuya pendiente fue considerada, junto con los otros parámetros, para la optimización del proceso. Hasta nuestro conocimiento, aunque se habían realizado trabajos sobre la optimización del secado de zanahoria (Mulet, 1994; Zielinska y Markowski, 2010; Aghbashlo y col., 2010; Frías y col., 2010a) este es el primero en el que se emplearon como variables dependientes los 2-FM-AA, la vitamina C y el ß-caroteno, así como la pendiente del período de velocidad constante de secado y la pérdida de humedad durante la primera hora como variables del proceso. Los resultados obtenidos permitieron establecer las condiciones de secado más adecuadas para minimizar la pérdida de calidad y maximizar la eficiencia del proceso.

Una vez que conocimos los intervalos de temperaturas y tiempos con los que se podía trabajar en el equipo de secado por convección se decidió aplicar los conocimientos adquiridos al secado de fresas (Apartado 4.1.1.2.2). Como se ha indicado la fresa es una fruta de gran aceptación por su palatabilidad, baja concentración de carbohidratos y elevado contenido en vitamina C (60 mg/100 g producto fresco), entre otros constituyentes. En este caso, se realizaron ensayos a diferentes temperaturas y tiempos y se estudió la cinética de la pérdida de vitamina C, ajustándose a una cinética de orden uno, de modo similar a lo encontrado para otros sustratos deshidratados distintos a la fresa (McMinn y Magee, 1997; Khraisheh y col., 2004; Erenturk y col., 2005; Sanjinez-Argandoña y col., 2005, Goula y Adamopoulos, 2006; Orikasa y col., 2008). A pesar de ser una fruta muy estudiada, no existían trabajos previos en la literatura sobre la cinética de pérdida de vitamina C en el proceso de secado. Además, se determinó que la cinética de formación de los 2-FM-AA, detectados por primera vez en fresa deshidratada, era de orden cero, al igual que la determinada para la furosina en otros productos como los derivados del tomate (Hidalgo y Pompei, 2000). La dependencia de ambas reacciones frente a la temperatura se estableció mediante la ecuación de Arrhenius y se calcularon los correspondientes valores de energía de activación, los cuales fueron del orden de los reportados para otros sustratos vegetales (Lee y Labuza, 1975; Denninson y Kirk, 1978; Hidalgo y Pompei, 2000; Orikasa y col., 2008). Sin embargo, no se consideró la dependencia de ambas reacciones con la humedad, ya que los cambios más importantes que se observaban en el contenido en vitamina C y

en la formación de 2-FM-AA se producían a partir de las 3 h de tratamiento, cuando apenas existían modificaciones en la humedad. Ambos indicadores se correlacionaron mediante una regresión lineal simple, encontrándose valores del coeficiente de correlación superiores a 0,96. De los resultados obtenidos en este estudio se pudo comprobar una buena sensibilidad de ambos parámetros de calidad durante el secado de fresa, siendo la sensibilidad de formación de los 2-FM-AA superior a la de degradación de vitamina C, dependiendo ambas de la temperatura y del tiempo de procesado.

Dado que no sólo la etapa de secado afecta a la calidad final de vegetales y frutas deshidratados fue necesario conocer cómo influyen en dicha calidad los pre-tratamientos, entre los que cabe destacar el escaldado. En general, el escaldado se realiza, para inactivar enzimas que pudieran ocasionar un deterioro importante del producto a lo largo del período de almacenamiento, durante su vida útil. Por ello, en la siguiente fase del trabajo resultó de interés realizar un estudio sobre la eficacia de diversos pre-tratamientos, tanto convencionales como con US, en la inactivación de la POD y la PME y en las pérdidas por lixiviado durante el escaldado de zanahoria (Apartado 4.2.1.1). En los tratamientos con US apenas se encontró inactivación enzimática en los ensayos llevados a cabo en baño y con sonda a bajas temperaturas. Considerando los tratamientos con sonda de US, los valores más elevados de inactivación enzimática (90 y 50% para POD y PME, respectivamente) se consiguieron mediante los tratamientos con generación de calor durante 15 min, en los que se alcanzaban temperaturas de hasta 70 °C. Los resultados obtenidos demostraron el efecto combinado de los US y la temperatura y subrayaron la dificultad de identificar los mecanismos de inactivación enzimática debidos a la aplicación de US, ya que intervienen diversos efectos mecánicos y químicos derivados de la cavitación. Además, existen numerosos factores extrínsecos e intrínsecos a los parámetros del proceso que pueden incidir provocando activación o inactivación enzimática (Cruz y col., 2006; Chandrapala y col., 2012).

De los resultados obtenidos en este trabajo pudo inferirse que los US aplicados mediante sonda son especialmente adecuados como alternativa a los tratamientos convencionales LTLT, en los que se persigue la obtención de productos con menores modificaciones texturales. Así, se obtuvieron resultados semejantes de inactivación enzimática y pérdidas por lixiviado con

el tratamiento LTLT a 60 ºC durante 40 minutos y con sonda de US durante 10 minutos a temperaturas de hasta 60 ºC. En este último caso, la utilización de la sonda dio lugar a una reducción del 75% en el tiempo de pre-tratamiento, lo cual podría representar un ahorro energético importante. Sin embargo, teniendo en cuenta la inactivación enzimática, de todos los pre-tratamientos ensayados los más eficaces fueron los efectuados con vapor y con agua a ebullición durante tiempos cortos (HTST), que son los que se utilizan con mayor frecuencia en la industria.

Entre todos los pre-tratamientos, se eligieron los que provocaban las mayores inactivaciones enzimáticas con la menor pérdida posible por lixiviado. Posteriormente, las zanahorias escaldadas se deshidrataron en el prototipo de secado por convección bajo las condiciones óptimas previamente determinadas (46 °C y 4.9 m/s), evaluándose los efectos de los pre-tratamientos en la calidad final del producto deshidratado (Apartado 4.2.1.2.1). De todos los indicadores estudiados (2-FM-AA, carbohidratos, polifenoles totales, vitamina C, perfil proteico, propiedades de rehidratación, cambios microestructurales) los resultados más relevantes se obtuvieron en los 2-FM-AA. Se pudo observar que las condiciones de escaldado a las que se somete el producto afectan especialmente a la formación de los 2-FM-AA durante la etapa posterior de secado, debido probablemente a modificaciones en la estructura de la proteína durante el pre-tratamiento. En concreto, las muestras de zanahoria deshidratada sometidas a un tratamiento previo con US presentaron niveles relativamente altos de 2-FM-AA, a pesar de las bajas temperaturas y los cortos tiempos empleados en los pre-tratamientos; por tanto, los efectos mecánicos provocados por los US pudieron ser la principal causa de dichas modificaciones estructurales. Así, se ha visto que los US pueden facilitar la exposición de zonas hidrofílicas de los aminoácidos, favoreciendo su interacción con los carbohidratos reductores durante las primeras etapas de la RM (Kresic y col., 2008; Mu y col., 2010).

Otras de las cualidades importantes a considerar en la calidad de los productos deshidratados son sus características organolépticas (Apartado 4.2.1.2.2). Para llevar a cabo la evaluación sensorial de estos productos las zanahorias se rehidrataron, ya que la aceptabilidad general aumenta y, además, el consumo habitual de los productos vegetales deshidratados es tras un proceso de rehidratación en un medio líquido (Lin y col., 1998). De

esta forma, en las muestras de zanahoria sometidas a diferentes tipos de pre-tratamiento y secadas por convección, se realizó una valoración de su calidad sensorial por parte de un panel de catadores semientrenados, y se encontró que las muestras de zanahorias pre-tratadas con US presentaban unas propiedades organolépticas adecuadas y similares a las muestras escaldadas por métodos convencionales. Como estudio complementario se planteó una diferenciación de muestras de zanahorias deshidratadas, en base a los pre-tratamientos aplicados, estudiando las huellas espectrales másicas obtenidas tras los análisis mediante Head-Space ChemSensor System (pseudonariz electrónica). Así, muestras de zanahorias que no habían sido diferenciadas por el panel de catadores fueron clasificadas en diferentes grupos mediante esta metodología, indicando, por primera vez, su utilidad en la diferenciación de zanahorias procesadas bajo condiciones similares y/o con composición semejante.

Una vez estudiado en profundidad el proceso de secado convectivo, en la fase final de la presente Memoria se aplicaron los US para deshidratar zanahoria y fresa como posible alternativa o complemento a los procesos por convección (Apartado 4.3.). En la búsqueda de procesos de deshidratación eficientes varios trabajos ya habían destacado los resultados prometedores de la aplicación de US de potencia (De la Fuente-Blanco y col., 2006; Chemat y col., 2011; Cárcel y col., 2012) debido, principalmente, a su capacidad para acelerar el proceso de eliminación de agua sin someter a los alimentos a temperaturas elevadas de procesamiento. Sin embargo, hasta el momento, no se habían llevado a cabo investigaciones sobre la calidad y bioactividad de los productos resultantes. Por lo tanto, en primer lugar, se realizaron ensayos de deshidratación de zanahoria en un equipo de US de potencia por contacto y se estudió la calidad global del producto final (Apartado 4.3.1.1.). En general, se detectó un escaso avance de la reacción de Maillard y, en base a la mayor parte de los indicadores estudiados, se comprobó que la calidad de las zanahorias tratadas con US era semejante a la de las zanahorias liofilizadas en el laboratorio.

A continuación, se procesó fresa en un equipo de secado asistido por US de potencia sin contacto, estudiándose, además de la calidad, la cinética de pérdida de humedad mediante un modelo difusional teniendo en cuenta la resistencia externa a la transferencia de materia y el encogimiento

(Apartado 4.3.1.2.1.). Se identificaron la difusividad efectiva (D_e) y el coeficiente de transferencia de masa (k), observándose un incremento de los mismos por la aplicación de los US, lo cual indicaba la aceleración del proceso por efecto de los US, de modo similar a lo que previamente se había encontrado en la literatura para otros sustratos (García-Pérez y col., 2006a; Ortuño y col., 2010; Ozuna y col., 2011; Cárcel y col., 2011). En las muestras de fresa deshidratada también se determinó la pérdida de vitamina C, la formación de 2-FM-AA y las propiedades de rehidratación (Apartado 4.3.1.2.2). De los resultados obtenidos, se concluyó que la aplicación de US de potencia no sólo reducía el tiempo de secado entre un 14 y 33% sino que mantenía una elevada calidad del producto final, de acuerdo a los parámetros químicos y físicos estudiados. Es de destacar que, incluso en las condiciones más enérgicas (70 °C, 60 W), las muestras de fresa presentaron valores elevados de retención de vitamina C (> 65%) y escaso avance de la RM. Se comprobó también la seguridad microbiológica del producto a lo largo de un período de almacenamiento de 6 meses a temperatura ambiente. Además, se estudió la evolución de la vitamina C durante dicho período de conservación, observándose similares pérdidas en dicha vitamina C (próximas al 50%) en las muestras deshidratadas con US y sin US al final del período de almacenamiento.

Según los resultados hallados puede considerarse que, de modo global, las muestras de zanahoria y fresa deshidratadas bajo el efecto de los US mostraron poseer una calidad superior a la de muestras comerciales. Dependiendo de los indicadores empleados, dicha calidad fue equivalente a la de muestras secadas por convección en similares condiciones e incluso, en ciertos aspectos, a muestras liofilizadas.

A través de los resultados obtenidos en la presente Memoria se persigue ofrecer una amplia y novedosa información sobre la deshidratación de vegetales y frutas para contribuir al conocimiento sobre la elaboración de alimentos de calidad, seguros y saludables y así, satisfacer las necesidades del consumidor actual, tal y como se planteó en los objetivos iniciales. El trabajo aquí desarrollado ha pretendido ser multidisciplinar, abarcando diferentes aspectos de interés en Ciencia y Tecnología de los Alimentos relacionados con la deshidratación de alimentos. A lo largo de esta Tesis se han empleado no sólo diferentes tipos de procesado (convencionales, por US)

y técnicas analíticas para la determinación de parámetros de calidad químicos y físicos, sino también métodos desde los más sencillos hasta más complejos (Matlab) de tratamiento de datos, lo cual ha contribuido a dar una visión global sobre la viabilidad de la aplicación de US en el procesado de vegetales y frutas. Sin embargo, a pesar de lo prometedor de los resultados, es preciso continuar en el futuro en esta línea para poder llegar a instaurar los US como una tecnología de elección en la deshidratación de alimentos.

En general, las investigaciones actuales en el campo de la sonoquímica están dirigidas a la mejora y el diseño de sistemas ultrasónicos (generadores, reactores, transductores) con el fin de escalar esta tecnología y adaptarla a los procesos requeridos por las industrias de alimentos (Gallego-Juárez y col., 2010). Cabe destacar que el desarrollo de reactores de cavitación para el tratamiento en flujo continuo ha contribuido a introducir la aplicación de US en medios líquidos a gran escala. Actualmente, en la industria, ya se aplican US a procesos de homogenización, emulsificación y extracción (Soria y Villamiel, 2010). Por el contrario, en la aplicación de US en medios sólidos, son necesarias más investigaciones en la optimización de los procesos, tanto para los equipos por contacto como sin contacto, con el fin de desarrollar procesos más eficientes y obtener productos de máxima calidad.

Por otro lado, restan muchos estudios por realizar para desvelar los mecanismos exactos que ejercen los US sobre el tejido vegetal durante la deshidratación. Al día de hoy se sabe que los materiales más porosos son más propensos a experimentar el efecto esponja provocado por los US (García-Pérez y col., 2009; 2012a; Puig y col., 2012) y que la obtención de productos de elevada calidad es debida, fundamentalmente, a la aceleración del proceso de secado. Los estudios más recientes están enfocados a evitar la incorporación de energía térmica al proceso de deshidratación para que las pérdidas de compuestos termolábiles sean mínimas. Es el caso de la aplicación de US al proceso de liofilización (García-Pérez y col., 2012b; Schösler y col., 2012a). En este campo quedan aún por llevarse a cabo muchos estudios, empezando por la optimización las condiciones de proceso que permitirían preservar los compuestos nutritivos y/o bioactivos de la materia prima. En paralelo, son muy importantes los estudios de eficiencia energética y de evaluación de costes del proceso (pre-tratamiento y deshidratación) que permitirían atraer la inversión de la industria alimentaria

hacia la aplicación de estas tecnologías emergentes a procesos de deshidratación industrial.

Conclusiones

6. CONCLUSIONES

- Atendiendo al contenido en vitamina C y a la formación de 2-FM-AA, la calidad de las frutas deshidratadas del mercado es mejorable; particularmente, en aquellas que se supone fueron sometidas, a una deshidratación osmótica previo al secado convectivo.

- Mediante un análisis por RSM se han correlacionado con gran precisión las condiciones de operación con los parámetros cinéticos (pendiente del período de velocidad constante, pérdida de peso en la primera hora) y de calidad (2-FM-AA, vitamina C, β-caroteno) durante el secado de zanahoria por convección, encontrándose las condiciones óptimas de temperatura (46 °C) y velocidad del aire (4,9 m/s) para obtener un producto de alta calidad mediante un proceso eficiente.

- Durante el secado de fresa por convección la pérdida de vitamina C se ha ajustado a una cinética de orden uno y la formación de 2-FM-AA, detectados por primera vez en fresa, a una cinética de orden cero, encontrándose, de acuerdo al valor de E_a, una mayor sensibilidad de estos últimos como indicadores de proceso.

- Teniendo en cuenta la inactivación de la POD y las pérdidas por lixiviado, los pre-tratamientos de zanahoria llevados a cabo con sonda de US a temperaturas de hasta 60 °C podrían constituir una alternativa a los tratamientos convencionales LTLT, ya que se observan efectos similares pero en tiempos muy inferiores (75% menos).

- Las condiciones de escaldado a las que se somete el producto afectan significativamente a la formación de los compuestos de Amadori durante la etapa posterior de secado, subrayando la utilidad de los 2-FM-AA como indicadores del deterioro causado durante el secado convectivo de zanahoria.

- En muestras de zanahoria secadas por convección, las pre-tratadas con sonda de US presentaron una buena calidad sensorial, similar a las escaldadas convencionalmente mediante tratamientos LTLT y HTST. Además, el análisis estadístico de las huellas espectrales másicas, obtenidas mediante un sistema de pseudonariz electrónica o ChemSensor, permitió la diferenciación de muestras con similar composición secadas por convección y

pre-tratadas por diferentes métodos (US y convencionales), indistinguibles para el panel de catadores.

- La aplicación de US de potencia por contacto durante la deshidratación de zanahorias (100 W; 20-60 °C; 1.2 m/s) permitió la obtención de un producto de alta calidad con escaso avance de la RM y contenido en polifenoles, actividad antioxidante y perfil proteico similar a muestras liofilizadas.

- Mediante un modelo difusional que consideraba la resistencia externa y el encogimiento se comprobó que la aplicación de US de potencia sin contacto (30 y 60 W) durante el secado de fresa por convección (40-70 °C), producía una significativa mejora en la difusividad efectiva y en el coeficiente externo de transferencia de materia, respecto al mismo proceso sin asistencia de US, lo que se tradujo en una significativa reducción en el tiempo total de procesado.

- Atendiendo a la retención de vitamina C, a la formación de 2-FM-AA y a la capacidad de rehidratación, la calidad de fresas secadas en un proceso por convección asistido por US (30 y 60 W; 40-70 °C) fue superior a la de muestras de fresas comerciales y similar a la de muestras sometidas al mismo tratamiento pero sin US, en tiempos significativamente menores, indicando, todo ello, la idoneidad de esta tecnología, rápida y respetuosa con el medio ambiente, para la obtención de fresas deshidratadas de calidad.

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